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3700

Skin Transplantation as a Means of Analyzing Factors in Production and Growth of Feathers.

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In most breeds of fowl there is a well marked sexual dimorphism in the color and structure of adult feathers. Experimental or pathological alteration of the endocrine balance has been shown[1] to produce striking reversals and modifications in these characters. Our problem has been to find a new method of attack which would yield further information on (a) the extent to which the output of a feather follicle is controlled by the endocrine relations which obtain at the time the feather is produced, and (b) the extent to which it is determined by characteristics which are inherent in the follicle itself. The procedure has been to transplant pieces of skin from one newly hatched chick to another and, some months later, to compare the feather production of the donor, the host and the skin originally transferred from the former to the latter.

The technique is simple. Young chicks were found to take the anesthetic (ether) very satisfactorily. They recover quickly and do not suffer from infections when moderate care is exercised. The grafts are easily held in place by interrupted silk sutures, which usually fall out spontaneously, but may occasionally remain and serve as good landmarks over a period of several months. The general results obtained thus far may be briefly summarized.

1. The percentage of *initial* "takes" is high, but many grafts after having become well established are subsequently lost. Such grafts often appear entirely successful at first, and produce normal pin feathers or even fully developed feathers. The cause of these failures has not been studied, but the distribution of cases with reference to sex and breed suggests varying degrees of incompatibility between donor and host. This type of experiment promises to be very favorable for the study of differences in tissue reactions and "individuality differentials."

2. In the wholly successful cases the grafted skin has remained normal throughout (the older specimens are now adult), increased to many times its original size and produced a full complement of feathers, regularly replacing those which were shed or pulled out. In some instances the grafted skin seemed to function more vigorously than that of the host.

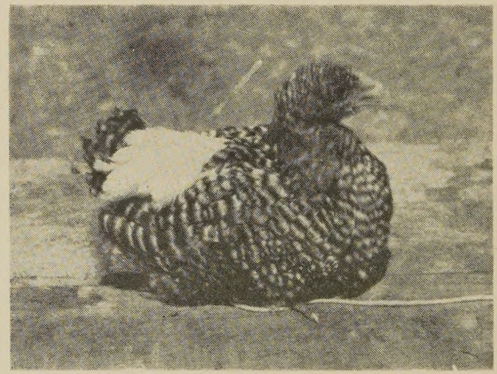

FIG. 1.
A Plymouth Rock chicken with graft from a White Leghorn.

3. The racial characteristics of feathers produced by grafted skin are those of the donor. Thus a bit of Rhode Island Red skin transplanted to a White Leghorn chick produces in due time feathers which are typically Rhode Island Red in color and texture. White Leghorn skin on a Plymouth Rock host produces feathers indistinguishable from those produced by normal White Leghorn fowls. Skin from a barred Plymouth Rock grafted on a Rhode Island Red produces typically barred black and white feathers. Yellow, black and penciled feathers have been produced by grafts from breeds of corresponding colors. Several months after the transplantation of the skin, follicles still continued to produce successive feathers typical of the breed to which the donor belonged. It may, therefore, be inferred that the white of the Leghorn or the barring of the Plymouth

Rock is due to factors inherent in the follicles themselves, rather than to special endocrine or general metabolic peculiarities of the breed. (Two cases with a few mosaic, black and white feathers in Rhode Island Red grafts on White Leghorn hosts are as yet unexplained. Since both of the donors died young, the purity of their own plumage cannot be vouched for.)

4. In regard to sex characteristics, on the other hand, feathers produced on the graft follow the host rather than the donor. For example, skin from a Rhode Island Red male grafted on a White Leghorn female produces feathers similar to those of a Rhode Island Red hen. Stated briefly: Feathers produced by grafts follow the breed of the donor, but the sex of the host.

5. The rate of feathering, or age of appearance of pin-feathers, which varies with different breeds, is largely influenced by the donor. Rhode Island Red skin grafted on a Leghorn develops pin-feathers more slowly than does the skin of the more precocious host, while Leghorn skin on a Rhode Island Red or Plymouth Rock produces pin-feathers earlier than the host.

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[1] Especially Cole, Crew, Domm, Goodale, Morgan, Pezard, Riddle, Torrey and Horning. There is a good summary of the literature in the paper by L. V. Domm, *J. Exp. Zool.*, 1927, xlviii, 15-173; and also in the paper by Frank R. Lillie, *ibid.*, 175-196.

3701

A Formula Expressing a General Relationship Between Blood Pressure and Body Weight.

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The question of a relationship between body weight and blood pressure may be studied from the findings in large numbers of individuals of various sizes essentially normal, particularly as regards the cardiovascular system and nutritional status. Children afford the best material for such a study. In them a large range of body size is available, and arterial disease is so rare that in group studies it can safely be disregarded as a disturbing factor. Satisfactory tables of normal weight or height are at hand for the exclusion of nutritionally unsuitable cases. By utilizing group averages the effects of temporary or accidental variations in pressure, of observational error, and of individual variations in body structure or composition can all be minimized.

The following study is based on observations of about 1,000 approximately normal children, between 4 and 16 years of age, mainly from kindergartens and public schools. By plotting individual blood pressures against body weights* it was found that the points of intersection roughly followed a curve of general parabolic form. Dividing the cases into twelve groups by age (without reference to sex) average weights and corresponding systolic and diastolic pressures for each group were determined, with the results shown in Table I. The logarithms of weight when plotted against the logarithms of pressure followed an approximately straight line, from which it was inferred that the relationship between weight and pressure could be expressed by the general formula $y=ax^b$, or $S/D=aW^b$. Solving for the six pairs and averaging, the following values for b were found: $b_S=0.2055$, $b_D=0.1900$. Assuming a uniform value of 0.2 for b, the average for the constant a was found to be $a_S=51.725$, or approximately 51.7, $a_D=34.730$, or approximately 34.7. The formulas derived for systolic pressure and diastolic pressure are therefore $S=51.7\ W^{0.2}$, $D=34.7\ W^{0.2}$, and by difference $P=17.0\ W^{0.2}$, in which S is systolic pressure, D is diastolic pressure, P is pulse pressure, all in millimeters of mercury; and W is body weight in kilograms.

The actual findings (group averages) of pressure are compared in the following table with the pressures calculated from body weights.

TABLE I.

Weight	Systolic pressure				Diastolic pressure			
	Found	Calc.	Difference		Found	Calc.	Difference	
Kg.	mm.	mm.	mm.	%	mm.	mm.	mm.	%
19.5	92.5	93.7	—0.8	0.9	62.5	62.9	—0.4	0.6
20.5	94.5	94.7	—0.2	0.2	64.0	63.5	+0.5	0.8
23.0	97.0	96.9	+0.1	0.1	65.5	65.0	+0.5	0.8
25.0	99.0	98.5	+0.5	0.5	67.0	66.1	+0.9	1.3
27.5	101.0	100.4	+0.6	0.6	68.0	67.4	+0.6	0.9
29.5	102.5	101.8	+0.7	0.6	69.5	68.3	+1.2	1.7
33.0	104.0	104.2	—0.2	0.1	70.0	69.9	+0.1	0.1
37.0	106.0	106.5	—0.5	0.3	71.0	71.5	—0.5	0.7
40.0	107.5	107.1	+0.4	0.2	72.0	72.7	—0.7	1.0
43.5	110.0	110.1	—0.1	0.1	73.0	73.9	—0.9	1.2
46.5	112.0	111.5	+0.5	0.4	75.0	74.9	+0.1	0.1
53.0	115.0	114.5	+0.5	0.3	76.0	76.9	—0.9	1.2
Average			±0.43	0.36			±0.61	0.87

In the following table the expected pressures are calculated for a wider range of body weight.

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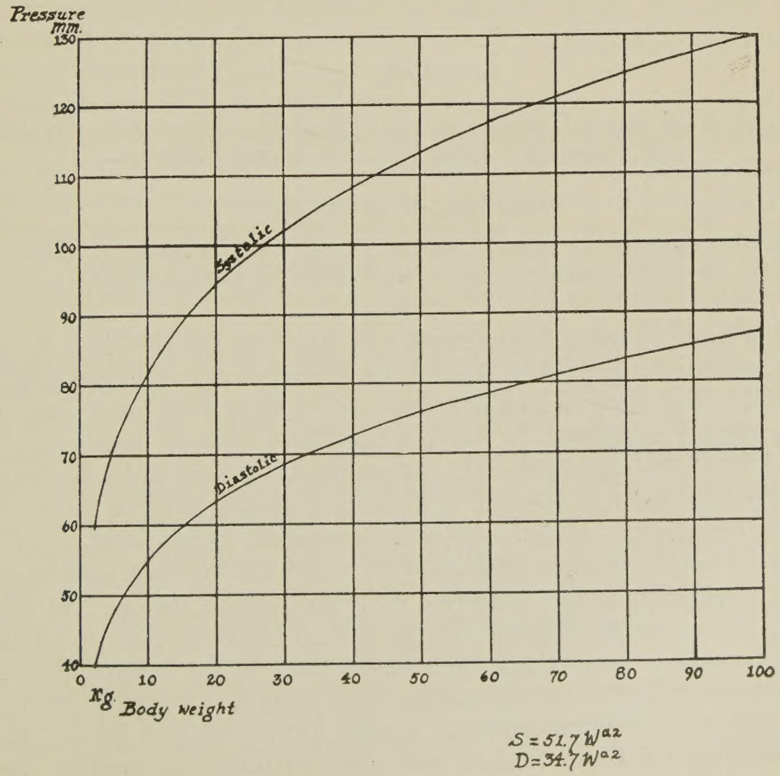
* The children were weighed clothed, but with coats and shoes removed.

TABLE II.

Weight	Systolic pressure	Diastolic pressure
kg.	mm.	mm.
2.0	59.5	39.9
3.0	64.5	43.3
3.5	66.5	44.6
4.0	68.3	45.8
5.0	71.4	47.9
10.0	82.0	55.0
20.0	94.5	63.5
30.0	102.2	68.6
40.0	108.2	72.6
50.0	113.2	75.9
60.0	117.3	78.7
70.0	121.0	81.2
80.0	124.3	83.5
90.0	127.3	85.4
100.0	130.0	87.2

The curves of the formulas are given in the accompanying graph.

Averages from the literature of some 25 observations of blood pressure in newborn infants (unfortunately nearly all by the pal-



patory method) gave a systolic pressure of 65.2 mm., and (from a smaller number of observations) a diastolic pressure of 41.6 mm. Since average birth weight is approximately 3.5 kg., the calculated pressures from the formula in newborn infants would be 66.5 mm. systolic and 44.6 mm. diastolic. The approximate average pressures in healthy adults of 70 kg. average weight are generally accepted as being 120 systolic and 80 diastolic. These are to be compared with 121.0 mm. and 81.2 mm. by the formula. The formula calls for 130 mm. systolic and 87.2 mm. diastolic at 100 kg. weight, but I have no normal data for comparison at this weight. It should be repeated that the formula postulates an average normal amount of body fat as essential to the correctness of the factors modifying body weight.

Until the normal variation has been determined the practical value of the formula as a standard of normal blood pressure in individual applications is unknown. At present it is offered merely as an expression of a general trend.

3702

Allergy Response to Tuberculosis in Guinea-Pigs Previously Treated with Tuberculin Fractions and Toxin Filtrates.

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The basis of these experiments is a study of the so-called Koch phenomenon elaborated by Krause[1] on allergy in experimental tuberculosis. The early anatomical response of the tissues of a susceptible animal inoculated for the first time with tubercle bacilli differs from that of a reinfection. In the susceptible guinea-pig a well circumscribed nodular tubercle will develop slowly after a week or more, without any of the signs of inflammation. Only later, after 3 or more weeks, does redness appear around the tubercle, associated with changes that are indicative of a progressive lesion. At this stage the animal has become allergic, *i. e.,* it begins to react in a new way because of the changes set up in the body by the tubercle bacilli. The susceptibility of the animal has been altered. If such an animal now receives an intracutaneous inoculation of tubercle bacilli, the response by the tissue is rapid and local inflammation occurs. Instead of well localized proliferation, diffuse exudation takes place. The inflammation occurs rapidly and subsides as rapidly, and the nodular tubercle now present retrogresses and may disappear completely. All of these changes are contingent, however, upon moderate dosages of tubercle bacilli.

In the first group of experiments, 5 series of normal guinea pigs, 3 animals in each series, were used. Subcutaneous injections of the different materials were given weekly in increasing doses over a period of 10 weeks, the initial dose ranging from 0.1 to 0.2 cc., and the final dose from 2.0 to 3.5 cc. Four weeks after the last injection, the animals were inoculated intracutaneously with 0.4 mg. of a moderately virulent strain of human tubercle bacilli. One group received tuberculin fractions*; another, tuberculin fractions and toxin filtrates* injected on alternate weeks; a third group received toxin filtrates alone; a fourth, the same toxin filtrate heated for one hour at 75° C.; and the fifth untreated group served as a control.

The local changes at the site of inoculation on a cleanly shaved area on the back of the animal could be observed from day to day. Attention was paid to the anatomical character of the lesion and the regional lymph node involvement. A record of the body weight and general condition of the animal was kept.

The results of these experiments were uniform and quite conclusive. Having previously noted the differences between a primary infection and a reinfection with tubercle bacilli in the normal animal, it was possible to compare these with the sequence of events in animals previously treated with toxic filtrates and tuberculin fractions injected alone or in combination. If allergy and specific resistance to tubercle infection can be developed artificially with such materials, then a subsequent inoculation of tubercle bacilli should be followed by the reactions that are characteristic of reinfection in a previously tuberculized animal. Such was found to be the case in these experiments. Normal guinea pigs thus treated and inoculated into the skin with tubercle bacilli showed a prompt local reaction noticeable within 48 hours, an early development of nodule in an inflammatory zone, and a lesion that came rapidly to a standstill and tended later to retrogress and heal. The regional lymph nodes did not show the rapid and pronounced enlargement peculiar to untreated control animals. In this instance, early localization and focal involvement were accomplished in exactly the same manner as that caused by a previous inoculation with tubercle bacilli. However, when guinea pigs had been previously injected with inactivated toxin filtrate, the animals behaved like untreated controls. In these the immediate reaction was uniformly absent; the nodule developed on an uninflamed background in 1 to 2 weeks. The lesion after this time advanced rapidly and approximated the condition seen in reinfected tuberculous animals at the time of reinoculation, and there was a later tendency to progression with softening and caseation.

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* Alcohol-insoluble fraction and ether-insoluble fraction prepared from culture of moderately virulent human tubercle bacilli grown in synthetic non-protein media. Toxin filtrate (Berkfeld) from 16 to 21 day old cultures of moderately virulent human tubercle bacilli grown in broth. 1 cc. of alcohol-insoluble fraction = 114 mg. tuberculin.

The most striking and characteristic picture was observed in the groups of animals that received tuberculin fractions alone and in those receiving toxin filtrate and tuberculin fractions in combination. The sharp response appears to be due to the specific bacillary element contained in the tuberculin fraction.

In the second group of experiments, 2 series of normal guinea pigs, including 4 animals in each group, were studied to determine the possible relation between the number of injections and the development of allergy. One set of animals received 2 subcutaneous injections of tuberculin fractions and the other received 4 at weekly intervals. Each group was controlled with an untreated series. In these experiments the results were identical with those previously described. The allergy was developed to a greater degree in those animals receiving the larger number of injections.

The application of Krause's technic in this and similar studies appears to be of definite value, particularly because the mechanism of resistance or immunity can be studied accurately without basing conclusions on dubious differences in viability of the infected experimental animal.

In all respects, regarding the local, regional and constitutional reactions as seen in the different groups of animals, the results justified the conclusion that the allergy phenomena were correlated with the development of specific resistance or immunity. Histopathologic studies confirmed the clinical findings. Two animals in each group were sacrificed 3 months after the inoculation with tubercle bacilli and sections of the lung, liver, spleen and lymph nodes were studied microscopically. The type of reaction seen in the tissues varied with the number of infecting bacilli and confirmed Baumgarten's[2] observations regarding the proliferation of only one type of cell in response to inoculation with few organisms. The structure of the tubercle invariably showed features of a tumor, and had none of the characteristics of inflammation. Although no attempt was made to produce absolute protection against the infecting organism, the observations indicate that definite resistance to the infection must have been developed, thus substantiating the grossly visible changes at the site of inoculation.

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[1] Krause, A. K., *Am. Rev. Tub.*, 1917, i, 65.

[2] Baumgarten, P., Ueber Tuberkel and Tuberkulose. 1. Theil. Die Histogenese des tuberkulösen Processes. Berlin, A. Hirschwold, 1885, (cited by Krause).

3703

Gonad-Stimulating Hormone of Anterior Pituitary and Heterosexual Ovarian Grafts.*

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The rôle of the gonadal-stimulating hormone of the anterior pituitary in the development and regulation of the genital system has been previously reported.[1, 2] That the same stimulation of the ovarian follicle can be effected in ovaries which have been grafted into both normal and castrated males is here reported.

A series of 4 male rats, 96 days of age, may be taken as a typical group. Two of these were castrated and the ovaries of 20-day-old rats were transplanted into the *recti abdomines* and anchored with silk sutures. Into the other 2 unoperated males, ovaries of 20-day-old rats were transplanted, one ovary into each testis. Eight daily transplants of the fresh pituitary of an adult rat were then given to one of the castrated and one of the normal hosts. The other pair received no transplants. The rats were autopsied on the ninth day of the experiment and the grafts removed and sectioned. The ovarian grafts in the recti muscles of the castrated animals were well vascularized, but considerable degeneration, evidently due to an inadequate circulation, had occurred in the untreated animal. In the graft of the animal which had received the pituitary transplants many primary follicles had disappeared, but a score of large follicles, of mature condition and with apparently normal ova, were found.

In the untreated animal of the second pair of this series, the transplanted ovary had been resorbed. In the treated animal there was a typical bilateral ovariotestis. The vascularization was unusually good, and the follicles, both primary and secondary, were numerous. Many large, apparently normal follicles in the graft were

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* Aided by a grant from the National Research Council Committee for Research in Problems of Sex.

like those in a normal adult ovary, with little atresia of the follicles or ova.

I found no indication of a mutual antagonism of the sex glands, as first stated by Steinach,[3] but unconfirmed by Moore[4] and others.

The formation of an ovariotestis, or an ovarian graft of any type, depends primarily upon the vascularization of the graft, but the development of the follicles of the graft depends upon the amount of the gonadal-stimulating hormone which is available to the ovary. Since the testis with its established circulation will utilize the hormone of the intact hypophysis of the host, additional hormone must be added by the glandular transplants to obtain the optimal follicular development in the grafted ovary. The uniform success of the earlier investigators in obtaining good follicular development in ovarian grafts in castrated animals is explained by the fact that the hormone of the normal, intact hypophysis of the animal will furnish sufficient stimulus to the follicle, provided that good vascularization of the graft has occurred. It is not implied that pituitary transplants have any influence on the primary vascularization of the grafts.

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[1] Smith, P. E., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 131.

[2] Smith, P. E., and Engle, E. T., *Am. J. Anat.*, xxxiv, No. 2.

[3] Steinach, *Arch. f. Entw. Mech.*, 1916, xlii, 307.

[4] Moore, C., *J. E. Z.*, 1921, xxxiii.

3704

Pregnancy Following Super-Ovulation in the Mouse.*

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The daily transplantation of the mammalian anterior pituitary into the adult albino mouse or rat results in the development of a greatly increased number of ovarian follicles,[1] and is followed by super-ovulation. The number of ova liberated by one ovary varies from 20 to 48, as determined by a study of serial sections of the uterine tubes.[2]

Daily intramuscular transplantations of 2 mouse pituitaries into adult female mice, beginning the first day of the dioestrum, resulted

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* Aided by a grant from the National Research Council Committee for Research in Problems of Sex.

in mating within 12 hours after the second treatment. The animals were killed on the ninth or tenth day of pregnancy. The number of uterine implantations found on the ninth or tenth day was between 19 and 29. Recovery of normal fetuses at this time has not been constant, because of failure of fertilization or implantation in some cases. Another group of similarly treated animals will be permitted to go to term.

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[1] Smith, P. E., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 131.

[2] Smith, P. E., and Engle, E. T., *Am. J. Anat.*, xxxiv, No. 2.

3705

Glycogen of the Edible Mussel, Mytilus Edulis, L.

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Whether or not all glycogens are identical has been investigated repeatedly,[1] but no general agreement has been reached. The report by Samec and Isajevic[2] that dog-liver glycogen contains 0.721% P_2O_5 suggests the possibility that the question of the identity of all glycogens may be solved by ascertaining their respective phosphorus contents. The purpose of the present investigation was to compare the phosphorus content of *Mytilus* glycogen with that of the glycogen examined by Samec and Isajevic.

Samec and Isajevic give no information concerning the method by which their glycogen was prepared. The *Mytilus* glycogen used in the present investigation was prepared by the method of Pflüger, as modified by Starkenstein and Henze.[3] In addition, it was repeatedly precipitated from slightly acidulated solutions. Difficulties were encountered in filtration so that the material was unavoidably exposed for a long time to strong KOH. The glycogen finally obtained had a P_2O_5 content of 0.1168% as determined by the method of Embden and an ash content of 0.25%. It was white and contained iron, calcium and phosphorus.

Certain investigators have obtained glycogen free from ash. The attempt was, therefore, made to remove the ash of *Mytilus* glycogen by electrodialysis with ultrafiltration in a Bechold-König apparatus, using a 10% acetic acid-collodion solution for the membranes. Dialysis was continued until the readings of the ammeter connected with the apparatus were constant, which took 4 to 5 hours. The dialyzate contained phosphorus and calcium. Samec and Isajevic also electrodialyzed glycogen and found that it separated into a sol and a gel layer, the material in the sol containing more than five times as great a percentage of P_2O_5 as the material in the gel. *Mytilus* glycogen did not separate into two layers in this way, perhaps because during dialysis the direction of the current was frequently changed. The solutions obtained could not be precipitated with alcohol until after the addition of a few drops of a 1% ammonium acetate solution. Three samples were prepared from the same original glycogen preparation. One sample had an ash content of 0.0827% and a P_2O_5 content of 0.0343%; another an ash content of 0.0467% and a P_2O_5 content of 0.0368%. A third had a P_2O_5 content of 0.0361%. The ash gave a test for iron due probably, as Gatin-Gruzewska[4] suggested, to the prolonged treatment with KOH, since even the best KOH contains a certain amount of iron.

The difference in the P_2O_5 content and in the behavior of the phosphoric acid in electrodialysis of *Mytilus* glycogen and of the dog-liver glycogen of Samec and Isajevic is not necessarily significant. It remains to be investigated whether or not prolonged treatment with KOH removes P_2O_5 from glycogen or changes glycogen itself. Until that has been ascertained, it is uncertain whether the observations here presented indicate that, as seems probable, dog-liver and *Mytilus* glycogen are in fact different or merely that the differences herein recorded are artifacts due to differences in methods of preparation.

By the method of Embden, inorganic H_3PO_4 can be precipitated in the cold in presence of organic H_3PO_4. An attempt was therefore made to determine, if possible, how much of the phosphorus in undialyzed glycogen was organic and how much inorganic. No difficulty was anticipated, since phosphorus was readily detected by Embden's method in the dialyzate. Nevertheless, in solutions of undialyzed glycogen it was impossible to obtain any trace of strychnine phosphomolybdate precipitate, though 70% of the phosphorus present was capable of being removed by electrodialysis. The writer is not yet prepared to interpret this phenomenon.

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[1] Harden, A., and Young, J. W., *J. Chem. Soc.*, 1902, lxxi, 1224; Noris, R. V., *Biochem. J.*, 1913, vii, 26; Irvine, J. C., and Gilchrist, H., Food Investigation Board Report, Dept. Sci. Ind. Research, Gt. Brit., 1924, p. 63.

[2] Samec, M., and Isajevic, V., *Compt. rend.*, 1921, clxxii, 1079.

[3] Starkenstein, E., and Henze, M., *Z. physiol. Chem.*, 1912, lxxxii, 417.

[4] Gatin-Gruzewska, Z., *Arch. ges. Physiol.*, 1904, cii, 569.

3706

Manometric Method for Quantitative Determination of Lactic Acid.

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Van Slyke and Neill[1] have recently introduced an apparatus for the determination of blood gases by manometric methods. This manometric blood-gas apparatus has been used by Van Slyke and Sendroy[2] in determining blood Ca. The Ca is precipitated as the oxalate, and this is oxidized in the apparatus by the addition of acidified $KMnO_4$. The oxalate is oxidized to CO_2 and H_2O, and the CO_2 so produced determined in the usual way.

A modification of the Clausen method for the determination of small amounts of lactic acid was described recently by Friedemann, Cotonio, and Shaffer.[3] These investigators found that the use of manganous salt increases the rate and yield of aldehyde when lactates are oxidized by acid $KMnO_4$.

The writers have developed a method for the determination of lactates using the manometric apparatus of Van Slyke and Neill, and in part the modified Clausen reagents of Friedemann, Cotonio, and Shaffer.

In selecting a mixture of $MnSO_4$ and $KMnO_4$ that would fully oxidize lactic acid to acetaldehyde and CO_2, an excess of $MnSO_4$ was found to prevent the oxidation or greatly to retard the process and this was also true for equal amounts of $MnSO_4$ and $KMnO_4$. When excess $KMnO_4$ was present good results were obtained over a considerable range of ratios of $KMnO_4$ to $MnSO_4$ as shown in Table I. Shaffer gives the oxidation-reduction potential necessary to oxidize lactic acid as +1.36 at 25° C. but carries out oxidation at the boiling point, *i. e.*, at an Eh of approximately +1.74. The Eh of our oxidizing agent was about +1.44 in the concentration of sulphuric acid employed, namely Normal and at 20°.

TABLE I.

Oxidizing agent used		Eh at pH and temp. of exp.	Millimols of CO_2 found	Millimols of lactic acid used
cc. M/10 $MnSO_4$	cc. M/10 $KMnO_4$			
1.8	1.2	+1.425	4.99	8.70
1.5	1.5	+1.435	6.85	8.70
1.2	1.8	+1.438	8.36	8.70
1.0	2.0	+1.440	8.39	8.70
0.8	2.2	+1.441	8.42	8.70

Procedure: The apparatus was the closed manometer type of Van Slyke gas apparatus. Reagents consisted of 5N H_2SO_4, M/10 $KMnO_4$, M/10 $MnSO_4$, Standard Lactic Acid, 5 N NaOH. The solutions need not be made up with care as great variations in the ratio of the oxidant and reductant affect the Eh very little. Solutions should, however, be carefully protected from dust which would yield CO_2 by oxidation. The Mn solutions were acidified with 1/20 vol. of Normal H_2SO_4 to drive off CO_2. The 5 N NaOH was made air free by evacuation and protected from air as described by Van Slyke and Neill. An approximately Normal Lactic Acid solution was made by dilution and filtered through acid hardened filter paper. This solution was made up to N/100 and titrated against N/100 carbonate free alkali with phenolphthalein as an indicator. During the titration CO_2 was removed by rapidly bubbling CO_2 free hydrogen through the closed titration vessel.

The Van Slyke apparatus was cleaned by evacuating and shaking with 2 cc. N H_2SO_4 and 5 cc. H_2O for a period of 2 minutes and ejecting the washings. One cc. of lactic acid containing 0.783 mg. was introduced near the bottom of the cup, the sides of the cup were washed down with 1 cc. 5 N H_2SO_4, and M/10 $MnSO_4$ and M/10 $KMnO_4$ were introduced. Each of the four solutions was drawn into the evacuation chamber separately. The total volume of solution amounted to 5 cc. The cup was then sealed with mercury, the mercury level in the evacuation chamber lowered to the 50 cc. mark and the instrument shaken for a period of 3 to 6 minutes. The gas was then carefully brought to 2 cc. volume by allowing the Hg to slowly rise and the pressure (p_1) was then read, amounting to about 110 mm. This process was repeated until 3 minutes of evacuation showed no change in pressure. Two cubic centimeters of 5 N NaOH were then introduced into the clean cup. The mercury was lowered to about the 15 cc. point, the lower stop cock of the apparatus closed, and the upper opened allowing the NaOH to enter. The instrument was shaken twice to mix the solutions and the Hg slowly allowed to rise to again reduce the gas to 2 cc. volume and p_2 was read. This usually amounted to about 89 mm. A correction (c) of 1 mm. to be added to p_2 was found by the method of Van Slyke and Neill. The volume of reagents having totaled 7 cc., Van Slyke and Neill's values in column 4 of Table III, page 544 (loc. cit.) may be used to calculate millimols of CO_2 found. The values must be multiplied by 2 as only 1 cc. samples were used in place of 2 cc. as in the table.

Rates of reactions involving gas formation can be very readily studied by this method. In the present instance the development of gas pressure may be measured at regular intervals and when plotted against time shows a beautiful monomolecular curve. The values for (p_1) in one oxidation, for example being 390, 458, 484, 498, 501, 500 in successive 3 minute intervals.

The agreement of determinations of lactic acid in a total quantity of less than one milligram was very good as shown by Table I. When oxidizing mixtures of the optimum proportions were used the values found agree within 1.0% of each other and 4% of the theoretical value. This compares favorably with any other method of determining lactic acid.

The application of this method to the determination of pure lactates is recommended but it should be pointed out that we obtained high values with silver lactate, probably due to the oxidation of acetaldehyde by silver compounds. A danger also lies in the presence of any other oxidizable compound yielding CO_2 on treatment with the reagents described above. The authors are now studying the use of the method with biological material.

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[1] Van Slyke, D. D., and Neill, J. M., *J. Biol Chem.*, 1924, lxi, 523.

[2] Van Slyke and Sendroy, J., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 167.

[3] Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, lxxiii, 335.

3707

The Blood of the Normal Guinea Pig.

WM. R. LYONS AND F. RENE VAN DE CARR. (Introduced by H. M. Evans.)

From the Anatomical Laboratories of the University of California.

It is suggested that inconsistencies in the work of previous investigators on the blood of the normal guinea pig have been due, for the most part, to the use of the usual fixed staining technic. The supravital method of blood study, first used by Pappenheim,[1] and reintroduced by Simpson,[2] overcomes the common difficulties of cell distribution, and identification, as met with in the smear method.

The peripheral blood of the normal guinea pig has been studied by the Neutral Red-Janus Green supravital method. Sixty-four guinea pigs from four different sources, of both sexes, and weighing from 500 to 1000 grams, comprise the series. From 200 to 400 white cells per preparation were counted on each. To compensate for normal fluctuations, counts were made at all hours of the day and night.

The normal guinea pig has been found to have a leucocytic formula peculiarly its own, especially in that an atypical mononuclear is normally present in the circulation. In our counts this will be termed the Kurlow cell, because of its inclusion, the so-called Kurlow body. Considerable evidence has been obtained upholding the belief that the Kurlow inclusion is the result of a symbiotic protozoan.

An average total leucocyte count of 9,600, and a total erythrocyte count of 5,565,000, were established. The differential count showed the following: Neutrophile, 34.9%; Lymphocyte, 49%; Monocyte, 7%; Eosinophile, 3.1%; Basophile, 0.8%; Kurlow cell, 5.0%; Unclassified cells, 0.2%.

It is believed that the above data establishes a fairly accurate means of ascertaining the normalcy of a fully matured guinea pig.

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[1] Pappenheim, *Virch. Arch.*, 1899, Bd., 1575.54, Anm.

[2] Simpson, M. E., Publications in Anatomy of the University of California, i, 1-9.

3708

"Tutocain" as a Shock Preventative in Tuberculous Guinea Pigs Injected with Tuberculin.

F. RENE VAN DE CARR AND WM. R. LYONS. (Introduced by H. M. Evans.)

From the Divisions of Bacteriology and Anatomy, University of California.

The symptoms following the rapid absorption of tuberculin in the tuberculous guinea pig, in which the subcutaneous inoculation of small amounts of tuberculin invariably prove fatal, are well known.

Fischel[1] has suggested that the severe tuberculin reaction can be mitigated by introducing tuberculin into the skin with a view to retarding absorption and slowly removing the toxic factors elaborated upon the contact of tuberculin with tuberculous tissues. It had occurred to us that if conditions could be set up subcutaneously which would retard absorption of this toxic factor, and at the same time permit the use of larger amounts of tuberculin, some therapeutic results might be developed, without the danger of fatal issue.

To produce these conditions, a local anesthetic, hydrochloride of p-amino-benzoyl-di-methyl-amino-methyl-butanol or by its trade name, the "Tutocain" brand of Butamin, which induces an edema and at the same time renders it painless, was selected. As a rule 4 cc. of a 1% solution was injected subcutaneously. The guinea pigs had been rendered tuberculous by the subcutaneous inoculation of 1 cc. of a suspension of tubercle bacilli (virulent strain No. 427) into the inguinal region 4 weeks previous to the tests. Animals were subjected to an intracutaneous tuberculin test before they were employed in the experiments, only animals showing a strongly positive reaction being used.

One half hour after the application of the drug 0.4 cc. of old tuberculin was injected subcutaneously into the infiltrated area. Previous tests had shown that 0.2 cc. of the same tuberculin was sufficient to cause death of tuberculous guinea pigs within 24 hours.

Guinea pigs, from numbers 1 to 10, were autopsied one week after the test inoculation and the extent of the infection noted. All guinea pigs showed extensive and typical tuberculous lesions in the liver, spleen, lymph nodes, and lungs. The remainder of the guinea pigs were kept under observation for subsequent tests.

TABLE I.

Guinea Pig No.	Inoculation	Reaction
1	Drug and 30 min. later 0.4 cc. old tuberculin	None except positive intradermal
2	”	”
3	”	”
4	”	”
5	”	”
6	”	”
7	”	”
8	”	”
9	”	”
10	”	”
11	”	”
12	”	”
13	”	”
14	”	”
15	”	”
16	”	”
17	”	”
18	”	”
19	”	”
20	0.4 cc. old tuberculin	Death in 14 hours
21	”	” 24 ”
22	”	” 16 ”
23	”	” 22 ”
24	”	” 18 ”
25	”	” 18 ”
26	4 cc. of drug and 0.4 cc. old tuberculin. Stood at room temperature for two hours. Inoculated simultaneously.	” 19 ”

The data shown in Table I indicate that tuberculous guinea pigs previously injected with Tutocain, and later with 0.4 cc. of old tuberculin, are afforded complete protection against a fatal dose of tuberculin. On the other hand, a simultaneous injection of the drug mixed with the tuberculin was fatal. This would appear to show conclusively that the Tutocain has no destructive effect upon the active substance of the tuberculin. In this connection it should be noted that the intradermal tuberculin reaction is not interfered with, even when the skin has been previously infiltrated with the drug.

Attention may be drawn to the possibility that the protective action of "tutocain" may not be directly dependent on its anesthetic properties, and in particular its effects on the autonomic system. The protection may be due to the oedema; in the protected animals, either a local mobilization of cells and ferments destroy the nucleoproteins of the tuberculin, or, the slow absorption favors a gradual but progressive desensitization of the animal, and so protects it from a fatal reaction.

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[1] Fischel, Carl, *Am. Rev. Tub.*, 1927, xvi, 210.

3709

Effects of Anesthetics on Osmotic Resistance of Erythrocytes: I. Ether and Chloroform.

C. D. LEAKE, CHARLOTTE BACKUS, HOBART BURCH AND KATHARINE O'SHEA. (Introduced by C. L. A. Schmidt.)

From the Pharmacological Laboratories of the University of Wisconsin and the University of California.

Widely conflicting opinion exists with regard to the effect of anesthetics on the resistance of red blood cells to hypotonic saline solutions. Since little of this is based on quantitative data, it was thought worth while to restudy the problem, using the quantitative method introduced by Simmel,[1] in which actual count is made of the number of cells remaining after uniform periods of contact with different grades of hypotonic solutions. The results are conveniently expressed as percentages of the total number of erythrocytes per cubic millimeter, where Hayem's solution has been used as the diluent. The normal variations likely to be encountered by this method have been studied by Leake and Pratt.[2]

Dogs were used in all experiments. Blood was drawn by syringe from the femoral artery, through the intact skin. Ether or chloroform was administered by the drop method, and further samples of blood were drawn at various intervals up to one hour after the induction of the surgical stage of anesthesia. These were prepared for determination as soon as drawn.

After about 10 minutes of ether anesthesia, an apparent increased resistance was noted on the part of the erythrocytes to the hemolysing action of the different strengths of hypotonic Simmel's fluid. This may have been due either (a) to destruction of weaker cells, leaving only the more resistant ones in the circulation, or (b) to an influx of cells, from such reservoirs as the spleen, resulting from struggling or partial asphyxiation during induction. Studies are in progress to determine which is the more likely. After 30 minutes of ether anesthesia, the osmotic resistance of the red cells was much below the normal level, indicating injury to the stroma of all cells following prolonged contact with the ether.

With chloroform, there was a prompt and marked lowering of the osmotic resistance of the erythrocytes, giving evidence of immediate damage to the stroma, both of cells already in circulation and of any which may have entered circulation after anesthesia was started.

Experiments *in vitro* are in progress.

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[1] Simmel, H., *Deutsch. Arch. f. kin. Med.*, 1923, cxlii, 252.

[2] Leake, C. D., and Pratt, H., *J. Am. Med. Assn.*, 1925, lxxxv, 899.

3710

Effects of Anesthetics on Osmotic Resistance of Erythrocytes: II. Nitrous-oxide or Ethylene with Oxygen.

C. D. LEAKE, HARRY LAPP, JANE TENNEY AND R. M. WATERS.
(Introduced by C. L. A. Schmidt.)

From the Pharmacological Laboratories of the University of Wisconsin and the University of California.

Nitrous-oxide or ethylene in combination with oxygen were administered to dogs by the rebreathing technique developed by Dr. Ralph M. Waters, in which the carbon-dioxide produced by the body is removed by soda-lime. Nitrous-oxide with oxygen caused a slight reduction in the osmotic resistance of the red cells to hypotonic Simmel's fluids, which even after an hour's anesthesia, was not nearly as marked as that noted under ether or chloroform. Ethylene with oxygen had about the same effect on the erythrocyte fragility. When ethylene was pushed to asphyxiation after 20 minutes without oxygen, the osmotic resistance of the red cells was reduced to a considerable extent in the lower dilutions of the hypotonic Simmel's fluid (0.4%), but in the higher dilutions (0.7% and 0.6%) it was only slightly below the normal levels.

3711

Effects of Anesthetics on Osmotic Resistance of Erythrocytes: III. Carbon-Dioxide with Oxygen.

C. D. LEAKE, HARRY LAPP, JANE TENNEY AND R. M. WATERS.
(Introduced by C. L. A. Schmidt.)

From the Pharmacological Laboratories of the University of Wisconsin and the University of California.

A mixture of 25% carbon-dioxide with 75% oxygen was administered to dogs without rebreathing. This greatly increased the

TABLE I.

Showing sample but typical effects of general anesthetic agents on the osmotic resistance of erythrocytes to hypotonic saline solutions as tested by Simmel's technique.

Anesthetic status of animal	Percentage of total red blood cells remaining after 1 hour's exposure to:			
	70% of isotonic Simmel's fluid	60% of isotonic Simmel's fluid	50% of isotonic Simmel's fluid	40% of isotonic Simmel's fluid
Dog 3: Normal, before anesthesia	90	73	36	30
15 minutes under ether	90	82	41	30
40 minutes under ether	78	66	28	8
Dog 11: Normal, before anesthesia	87	80	62	12
15 minutes under chloroform	84	58	23	3
35 minutes under chloroform	70	63	21	5
Dog 16: Normal, before anesthesia	94	66	53	22
20 minutes under nitrous-oxide administered with oxygen	88	59	44	12
Dog 19: Normal, before anesthesia	84	79	61	57
15 minutes under ethylene administered with oxygen	74	73	50	46
5 minutes later, during which interval 25% carbon-dioxide with 75% oxygen were administered	86	86	80	47

amplitude of respiration and induced anesthesia with abolition of the corneal reflex in 6 minutes. The pulse was greatly retarded. The mucous membranes remained pink. Clonic convulsions initiated by tremors came on within 10 minutes after induction, and violent tetanic convulsions quickly followed. Upon administering pure oxygen the convulsions promptly stopped, the pulse became very rapid, and the animals recovered. In all cases, except in the weaker dilutions (0.4%), the resistance of the erythrocytes to hypotonic Simmel's solutions was greater than normal.

After the administration of CO_2, much difficulty was experienced in obtaining blood because of the rapid clotting. Hickman noted this action of carbon-dioxide a century ago. Dr. Cline Chipman of Washington, D. C., has observed clinically that a few breaths of carbon-dioxide administered during the anesthetic period will greatly shorten the coagulation time of blood. This has been confirmed by Dr. R. M. Waters of Madison, Wis., and by Dr. M. E. Botsford and Dr. M. J. Price of San Francisco. Such an effect of carbon-dioxide may be of great clinical value in certain cases of capillary oozing at operation. Some phases of the relation of the CO_2 content of blood to its coagulation time have recently been studied by Baumberger.[1]

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[1] Baumberger, J. P., *Arch. Internat. Physiol.*, 1926, xxvii, 86.

3712

Are the Protozoan Faunae of Termites Specific?

S. F. LIGHT AND MARY F. SANFORD. (Introduced by C. A. Kofoid.)

From the Department of Zoology, University of California.

The species of the lower families of termites, so far investigated, have each a characteristic hindgut fauna of flagellate Protozoa, belonging to the orders *Hypermastigida* and *Polymastigida.* All individuals of a given termite species over wide geographic ranges present, so far as known, a similar grouping of species. This raises the question whether we are dealing here with specific protozoan faunae, whose species are able to exist only in the one specific termite host, with which they have established obligatory physico-chemical relations, or whether the faunae are distinct because of the isolated habit of life of the termite colony.

A colony of *Porotermes froggatti* received in normal condition, but which an examination of nearly 100 intestines showed to have lost its protozoan fauna, supposedly due to conditions encountered in transit, was used. Various methods were resorted to in attempts to refaunate the *Porotermes* individuals with the Protozoa from species belonging to other genera of termites.

All attempts to accomplish this by keeping *Porotermes* individuals in close association with *Termopsis augusticollis, Neotermes malatensis* and *Reticulitermes hesperus* proved failures.

Feeding defaunate *Porotermes* individuals with the intestinal contents of the 3 species named failed to accomplish transfaunation in most cases. In a few instances Protozoa survived for a few days, polymastigotes in all cases. No signs of increase of numbers or permanent establishment were noted.

All attempts to infect *Porotermes* individuals by applying to the anal aperture a drop of intestinal content of the infective species were failures.

Finally resort was had to injection by pipette through the anal aperture. For these experiments *Termopsis* was used as the source of infective material. Several cases of persistence for several hours and even a few days were observed, and finally with the last individual what seemed to be successful transfaunation was obtained. After 12 days an examination of the intestinal content showed the 4 larger members of the *Termopsis* fauna, *Trichonympha, Leidyopsis, Trichomonas* and *Streblomastix,* present in numbers and apparently normal in every way. The great numbers of *Trichomonas* suggested that this form had multiplied within the new host.

These experiments are reported in spite of their obviously preliminary nature, since the *Porotermes* stock was destroyed by Argentine ants and it seems improbable that more will be available. Experiments are in progress using the local species of termites to test the possibility of transfaunation, and to determine the normal method of refaunation within a species.

Illinois Branch.

Michael Reese Hospital, October 25, 1927.

3713

Modification of Adrenalin Effect with Functional Status of Stomach.

SERGIUS ARQUIN. (Introduced by W. F. Petersen.)

From the Department of Pathology and Bacteriology, University of Illinois, College of Medicine.

There has been a growing conviction among physiologists and pharmacologists that the reaction of an organ to stimulation will vary, within certain limits, with the state of tonus of the organ at the time of excitation. This conviction applies to drugs involving the autonomic nervous system, but by no means confined to them. A good part of the work has been done on strips of extirpated muscle, or the nerves of the organ have been cut, or numerous drugs influencing the autonomic system have been used in succession, so that, the results must be interpreted in the light of altered physiologic status.

Our work was done on a series of normal dogs trained to lie perfectly quiet under no restrain. A fine balloon at the end of a duodenal tube was passed by mouth (the dogs offer very little resistance after the first 3 or 4 times) and the duodenal tube was connected to a water manometer. The balloon was inflated with 100 cc. of air and the fluctuations of the manometer levels were recorded on a slowly revolving Kymograph. After a suitable control period an injection of 5 m. of 1 :1000 Parke Davis Adrenalin was given intracutaneously, in the leg, forming a wheal. (Intracutaneous injection was chosen because a mild relatively prolonged action was desired.)

Controls. The animals' gastric functions were established over a period of one to two weeks under daily observation. They showed: (1) That hunger contractions in a starving animal last from 30 to 50 minutes, and periods of active motility are separated from each other by periods of relative quiescence. (2) These periods of quiescence last from 1 to 1½ hours. In a few instances, however, they were as short as 45 minutes. (3) There was at no time a complete relaxation of the stomach in a starving animal. During the period of relative quiescence there were regular tonus waves occurring every 2 to 3 minutes. (4) For 3 to 4 hours after feeding, the stomach shows active digestive contractions, which lead directly into hunger contractions.

Results. The results obtained after the intracutaneous injection of adrenalin were as follows: (1) In a starving animal during the period of relative quiescence the administration of adrenalin intracutaneously resulted within 10 minutes after complete absorption of the adrenalin (as indicated by the disappearance of the wheal) in a rapid increase in gastric tonus and forcible hunger contractions. Usually the absorption of adrenalin would be complete 10 to 15 minutes after injection. (2) In a starving animal with active hunger contractions the administration of intracutaneous adrenalin resulted (after absorption) in a marked gastric dilatation, much greater than the physiologic inhibition observed in our controls. This inhibition lasted for 20 to 30 minutes, after which the stomach gradually returned to the former status. (3) A stomach in active digestive contractions is not influenced at all by the administration of adrenalin—even if the dose is increased to 15 m. intracutaneously or 10 m. intramuscularly. Apparently, therefore, adrenalin injection may be followed by diametrically opposed reactions on the part of the stomach musculature depending on the pre-existing status. This property is manifested, not only on isolated strips of gastric musculature, as has already been reported[1] but also on the intact organ of normal animals.

Most of the older observers explain this apparent paradox on the basis of a predominance in the tonus of either the sympathetic or the parasympathetic nerve group, resulting in variations in reaction to stimulation.[2] Schilf[3] concludes that there must be some change in the organ proper, not in the nerves, that causes this modification of reaction. Burn and Dale[4] attributed these phenomena to the variations in the state of tonus of the capillaries which in turn are controlled by the amount of histamine in the tissues.

None of the numerous theories is very conclusive. Until some very substantial proof is forthcoming no more theories should be given. We do feel, however, from these experiments, that the site of reversal must be in the organ cells, although the possibility of involvement of the terminal neuro muscular apparatus cannot be definitely eliminated.

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[1] McSwinney and Brown, *J. Physiol.*, 1926, lxi, 261.

[2] Cow, D., *J. Physiol.*, 1918, lii, 301.

[3] Schilf, E., *Klin. Wochens.*, 1927, vi, 193.

[4] Burn and Dale, *J. Physiol.*, 1926, lxi, 185.

3714

Gall Bladder Versus Sphincter Papillae.

EDWARD A. BOYDEN.

From the Department of Anatomy, University of Illinois College of Medicine.

An analysis of the contraction curves of the human gall bladder, constructed from cholecystograms taken at short intervals after a meal of egg yolk and cream, indicates that the average gall bladder discharges approximately ¾ of its volume in the first half hour after each meal. The first phase of contraction in which this is accomplished, is usually divided into three parts: the *"initial response"* (usually of 2 minutes duration), the *"2-minute pause"*, and the *"principal period of discharge"*. On the basis of Elman and McMaster's work on the sphincter of the dog,[1] the sudden discharge of the first 2 minutes is interpreted as being due to the opening of the sphincter; the interruption of emptying during the next 2 minutes to the initial increase in resistance offered by the sphincter to the flow of bile (as described by these authors); and the subsequent "principal discharge" to the contractile force of the gall bladder which, after slowly getting under way, is able for a time to overcome the growing resistance at the outlet of the common duct. That even the "initial response" (after ingestion of fatty foods) is partly due to contraction and not wholly to elastic recoil, is indicated by the change in shape which the human gall bladder undergoes during these first 2 minutes—that shape being then assumed which is characteristic of the rest of the first phase of contraction. (See also Ivy's demonstration[2] of the contraction and evacuation of the gall bladder caused by the intravenous injection of highly purified "secretin" preparations.)

Experiments designed to verify this action of the sphincter, show that stimuli other than the ingestion of food may provoke this "initial response". When a patient, for instance, is suddenly subjected to the odor of fried bacon, there ensues a momentry discharge of bladder bile (1 to 3 cc.)—comparable in duration to the initial drop in resistance of the sphincter described by Elman and McMaster, for in 2 minutes the gall bladder begins to fill again, in spite of the fact that the patient continues to smell food. The same initial response is elicited when a fasting patient swallows a glass of water, but frequently, in this case, reduction of the gall bladder continues from 12 to 35 minutes after the opening of the sphincter, and results in a total discharge of from 8 to 13 cc. of bile. It is not yet clear whether the presence of water in the duodenum inhibits the closure of the sphincter or merely accelerates the contraction of the gall bladder through the formation of a secretin-like hormone, in the way that water stimulates pancreatic secretion (Mellanby[3]).

Further light on the action of the sphincter is afforded by a fortuitous experiment upon a patient with an hour-glass gall bladder, in which the anatomy of the organ was such as to permit interpretation of its activity on the basis of the changing concentrations of its contents. This has established the fact that the human gall bladder may continue to contract in cases of shock even though the sphincter fails to open again after the "initial response." This is in accord with earlier experiments on laboratory animals which showed (1) that the gall bladder may continue to contract and to inject the entire biliary duct system during antiperistaltic obstruction of the common duct outlet[4] (l. c., plate 1); and (2) that even when the sphincter has been eliminated, the gall bladder may be made to contract by the ingestion of food[5] (l. c., Fig. 2) or by the injection of appropriate drugs[4] (l. c., plate 2).

From the above experiments it is apparent, however, that there are times when the resistance at the outlet of the common duct is too great to be overcome by the gall bladder, even though it continues to contract. This suggests that an explanation of individual and sex differences[6] in the emptying time of the human gall bladder may yet have to be sought for in the regulatory action of the sphincter papillae.

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[1] Elman, E., and McMaster, P. D., *J. Exp. Med.*, 1926, xliv, 151-171.

[2] Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, ...

[3] Mellanby, John, *Lancet*, 1926, xxi, 215-218.

[4] Boyden, E. A., *Anat. Rec.*, 1926, xxxiii, 201-256.

[5] Boyden, E. A., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 827-831.

[6] Boyden, E. A., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 383-385.

3715

Effect of Intravenous Injection of Dakin's Solution on Cholecystectomized Dogs.

J. P. BURGESS AND A. C. IVY.

From the Division of Physiology and Pharmacology, Northwestern University Medical School.

The production of cholecystitis by intravenous injection of a solution of chlorinated soda (Dakin's solution), was first reported by Mann.[1] He found that from 5 to 12 cc. per kg. body-weight, of a solution of chlorinated soda, having an available chlorine content of at least 0.48%, would produce a cholecystitis in dogs in 12 to 24 hours following the injection.

Since Dakin's solution seems to act specifically on the gall-bladder, its injection might prove fatal if the gall-bladder were removed, and, if so, the gall-bladder must act as the specific organ of elimination or detoxification. Such an assumption is justified by the observation of Mann, which we have confirmed, that the solution reaches the gall-bladder via the blood stream and not the bile.

Eleven normal dogs were injected intravenously with 10 cc. per kg. body-weight of a solution of chlorinated soda (Dakin's solution) containing 0.49% available chlorine. One dog died within 24 hours and the autopsy showed a cholecystitis. The other 10 were etherized after from 40 to 48 hours, and necropsies performed immediately. Of these, 3 showed normal gall-bladders, the remaining 7 all had cholecystitis of varying degrees of intensity. Several of these dogs had small hemorrhages beneath the serous coat of the liver adjacent to the gall-bladder; one having a large area of hemorrhage, 5 cm. x 2.5 cm.

Four healthy dogs on which cholecystectomy had been performed 3 weeks previously were then injected intravenously with 10 cc. per kg. body-weight of Dakin's solution containing 0.49% available chlorine. Two hours after the injection all the dogs were noticeably uncomfortable, and 3 of them were vomiting. All of these dogs were alive and apparently in good condition 3 days after the injection.

Because Doctors Shrager, Ivy and Kronenberg, in our laboratory had incidentally observed that death sometimes resulted when Dakin's solution was injected into dogs having a balloon in the gall-bladder; and because our cholecystectomized dogs all survived the injection of Dakin's, we thought that ether anesthesia might play some negative rôle in the elimination or detoxification of the solution. So, 3 days following the above injections, the cholecystectomized dogs were anesthetized with ether for from 15 to 30 minutes, and then allowed to recover. They were then given a second injection of like quantity of Dakin's solution containing 0.49% available chlorine. Forty-eight hours following this injection one dog died. The necropsy was entirely negative. The remaining 3 dogs were apparently unaffected by this injection, being in good condition 5 days later.

From these results, we conclude that the intravenous injection of Dakin's solution is somewhat more toxic in cholecystectomized dogs than in normal dogs.

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[1] Mann, *Ann. Surgery*, 1921, lxxiii, 54.

3716

The Source of Urinary Proteins.

EDMUND ANDREWS AND WM. A. THOMAS.

From the Department of Surgery, University of Illinois College of Medicine, Chicago.

It was previously reported by one of us[1] that complete picture of uremia in all its clinical, pathological and chemical details could be produced by the injection of hypertonic salt solutions into animals already made acid by the absorption of autogenous edema fluid. In these animals, although the kidneys remained histologically normal, there were evidences of degenerative changes in the liver. It was thought possible that the proteins in the urine in these experiments originated in the liver, and were the exciting cause of the renal changes which afterwards made the kidneys permeable to blood proteins. It is well known that foreign proteins injected into the blood are excreted almost quantitatively and that after a certain period the kidneys are thus rendered permeable to blood proteins as well.

Proteins from these artificial uremias and also those in the urine of dogs with ether nephritis were studied. Rabbits were sensitized to dogs' blood proteins by the subcutaneous injection of dog serum in the following series of doses: 1, 2, 4, 6, 8, and 10 cc. at 3 day intervals. The rabbits were sacrificed on the 21st day and bled. Sera thus obtained will react to dog blood proteins in dilutions of 1/1,000,000.

Urines were collected from our experimental nephritides in small 15 minute samples. Each sample was tested first for traumatic blood, which often spoiled the experiments. Next, all blood-free samples were tested for albumen and on all positive specimens, were tested against the serum. These tests were carried out both on whole urines and on urinary proteins, purified by dialysis and precipitation by ammonium sulphate saturation. From the earlier samples in a large series of experiments over 100 specimens were found in which the urinary proteins were free from serum proteins. With sera sensitive in dilutions of 1/1,000,000 they failed to react in dilutions of 1/10 and many times undiluted. Aside from the reports of Thomas[2] and a few others[3, 4, 5] these are the only blood-free urinary proteins ever studied. Previous attempts to ascertain the origin of urinary proteins by precipitin reactions have never been successful.

Next, normal dog livers were extracted, after perfusion until the perfusate no longer contained albumen. The liver was then ground in the ball-mill for 60 hours, extracted repeatedly with water, and the watery solution precipitated by saturation with ammonium sulphate. The precipitated protein was then freed of ammonium sulphate by dialysis. A serum was prepared by the above described method, which gave reactions in dilutions of 1/100,000 against liver protein. This serum was then matched against the urinary proteins (purified with urine), both from artificial uremias and ether nephritides, and precipitins reactions were again obtained in very high dilutions, at times as high as 1/100,000. This was controlled by taking samples of urinary protein which contained both blood and liver protein and mixing equal parts of urine and dog serum sensitized to dog blood. This was left over night, filtered, and then matched again against liver serum and reactions were obtained in just as high dilutions as before.

We are justified then in concluding that the albumens in the urine of certain types of nephritis originate in the liver.

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[1] Andrews, E., *Arch. Int. Med.*, 1927, xl, 548.

[2] Welker, Wm. H., Thomas, W. A., and Hektoen, L., *J. Am. Med. Assn.*, 1926, lxxxvi, 1333.

[3] Bramwell, B., and Paton, D. N., *Rep. of Lab. Roy. Coll. Phys. Edinburgh*, 1892, iv, 7.

[4] Bayne-Jones, S., Wilson, D. W., and Everett, H. S., *Bul. of Johns-Hopkins Hosp.*, 1923, xxxiv, 77.

[5] Hektoen, L., Kretschmer, H. L., and Welker, Wm. H., *J. Am. Med. Assn.*, 1924, lxxxiii, 1154.

3717

Growth Factor in Faulty Calcification in Experimental Obstructive Jaundice.

WILLIAM C. BUCHBINDER AND RUTH KERN. (Introduced by O. T. Schultz.)

From the Nelson Morris Fund, the John D. and Fannie K. Hertz Fund, the Nelson Morris Institute for Medical Research of the Michael Reese Hospital and the Department of Physiology of the University of Chicago.

Puppies subjected to common duct ligation and division, develop, in the state of late obstructive jaundice, a marked osteoporotic condition, in which are revealed roentgenographically cortical thinning, relatively wide medullary spaces, and a lack of film contrast generally. A marked lowering of the blood serum calcium accompanies this process. A slight lowering of the blood calcium first manifests itself after about 30 days of obstructive jaundice. Thereafter it is fairly uniformly progressive until it reaches values of between ⅔ to ½ the normal. Growth of skeletal structures, especially of the long bones, and a substantial increase in body weight occurs in the young animal with obstructive jaundice.

The normal blood calciums that obtain for the adult animal throughout the course of obstructive jaundice, where the same serious digestive disturbances arise, would speak against any defect in the capacity of the intestine to absorb calcium. The maintenance of normal blood serum calcium values in the early stages of jaundice in the puppy may be due to mobilization, *i. e.,* bone lysis. The four intrinsic factors in the production of faulty calcification are thought to be: first, small calcium storage; second, the presence of a disturbed metabolic condition unfavorable to osteogenesis; third, an intestinal factor characterized by the absence of some catalytic agent in bile; and lastly, new bone production which exceeds the rapidity with which lime salts are deposited.

The complete report of this paper is in press in the *Archives of Internal Medicine.*

3718

Prolongation of Life of Dogs with Experimental Intestinal Obstruction.

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From the Division of Physiology and Pharmacology, Northwestern University Medical School.

Harwell, Hoguet and Beekman,[1] in an experimental study of intestinal obstruction, produced obstruction of the duodenum in a series of dogs, and found that "the 3 dogs which lived the longest were all given saline solution subcutaneously." They injected from 150 to 300 cc. per day. In recent years numerous reports of the beneficial action of saline solution in gastric tetany and intestinal obstruction have appeared. We have undertaken a study of the effect of large quantities of saline solution on the length of life of dogs after the production of an experimental obstruction by the "closed loop" method introduced by Whipple and his associates.[2] We chose this method because it is a very uniform procedure, produces very characteristic results, and has been used widely by experimenters in this field. We have injected large quantities of normal saline solution, 500 cc. for each 7 kilos of body weight, 5 or 6 times a day subcutaneously, to provide fluid and chloride lost by vomiting and to cause a continuous diuresis. By this method we hoped to keep the dogs alive until the "closed loop" became relatively sterile,[3] or some other adaptation might occur.

Under ether anesthesia and using strictly aseptic technique, a "closed loop," 10 to 12 inches long, of the first part of the jejunum was made beginning 5 or 6 inches below the fixed portion of the duodenum and the continuity of the intestine was reestablished by an end-to-end anastomosis.

The length of life of the untreated dogs confirms the findings of Dragstedt, Moorhead and Burcky,[3] and others, namely that such dogs die in from 24 to 96 hours.

At the present time we have results on six treated dogs, which show that the treatment has proven efficacious. Dog 1 lived 86 hours and possibly should be excluded because of rupture of the loop at the line of closure. Dog 2 lived 102 hours (a very large dog). Dog 3 lived 151 hours. Dog 4 lived 163 hours. Dog 5 lived 242 hours (injections stopped 2 days before death). Dog 6 is now alive, 28 days after operation and has not been treated for the last 10 days.

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[1] Hartwell, J. A., Hoguet, J. P., and Beekman, F., *Arch. Int. Med.*, 1914, xiii, 717.

[2] Stone, H. B., Bernheim, B. M., and Whipple, G. H., *Bull. Johns Hopkins Hosp.*, 1912, xxiii, 159; Whipple, G. H., Stone, H. B., and Bernheim, B. M., *J. Exp. Med.*, 1913, xvii, 286.

[3] Dragstedt, L. R., Moorhead, J. J., and Burcky, F. W., *J. Exp. Med.*, 1917, xxv, 421; PROC. SOC. EXP. BIOL. AND MED., 1916, xiv, 17.

3719

The Influence of High and Low Protein Diets on Blood Chemistry.*

CHI CHE WANG, JEAN E. HAWKS AND AGNES A. WOOD.

From the Nelson Morris Institute for Medical Research of the Michael Reese Hospital, Chicago.

This paper is part of an investigation of the influence of high and low protein diets on basal metabolism, blood chemistry, and the urinary nitrogenous compounds. For a period of 37 days 6 apparently normal women, all engaged in laboratory work, received a high protein diet containing 2 gm. of protein and sufficient carbohydrate and fat to make 40 calories per kilo of body weight. An interval of 3 weeks was then allowed in which the protein intake was lowered until it reached 0.5 gm. per kilo. Although the carbohydrate and fat intake on the low protein diet was adjusted so that the caloric value of the two diets remained the same, all subjects lost weight during the first 10 days. The protein value was then increased to 0.6 gm. per kilo and the weight remained stationary. The low protein diet was continued for 30 days.

All foods were carefully weighed. On 4 days of each week basal metabolism was taken, specimens of blood and urine were collected, and samples of foods were saved and analyzed. Blood was drawn from the cubital vein without turniquet before breakfast, after 30 minutes rest, and was analyzed for sugar, total non-protein nitrogen, urea nitrogen, creatine, creatinine, uric acid, and lactic acid. Total non-protein nitrogen was determined by Koch's method and lactic acid by Clausen's procedure modified by Friedemann. Folin's methods were used for all the other constituents. All determinations were made in duplicate.

No consistent differences were found in blood sugar levels on the two diets. In 3 subjects the blood sugar showed a rise on the low protein diet, but in the other 3 it remained the same on both diets. The average figures for 6 subjects for the 5 weeks on the high pro-

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* Aided by the Gusta Morris Rothschild Fund, the S. J. T. Straus Fund, and the Otto Baer Fund for Clinical Research of the Michael Reese Hospital.

tein diet ranged between 86.9 and 80.9 mg. per 100 cc. of blood, while the corresponding values for the low protein diet were 91.6 and 83.5.

Lactic acid, on the other hand, was decidedly higher on the low protein diet. These values range from 11.1 to 20.7 mg. per 100 cc. of blood on high protein, and from 18.1 to 29.4 on low protein diet. Since lactic acid is one of the intermediary products of carbohydrate metabolism, it is possible that the increase in this constituent is due to the necessary high carbohydrate content of the low protein diet.

The high protein diet brought a marked rise in both total non-protein nitrogen and urea nitrogen in all 6 subjects. The values obtained on low protein diet are within normal range: 31.8 to 27.5 mg. per 100 cc. of blood for total non-protein nitrogen and 10.9 to 9.0 for urea nitrogen. On the high protein diet the total non-protein nitrogen ran between 57.2 and 37.0, and the figures for urea nitrogen were 20.6 and 13.0. A much greater variation in the same indiviual from week to week was found in both the total non-protein nitrogen and the urea nitrogen on the high protein diet.

Four subjects showed a slight rise in uric acid during the high protein diet. No difference was found in the other two. The figures on this diet ranged between 4.8 and 2.9 mg. per 100 cc. of blood. Those for low protein diet were between 3.9 and 3.1. The creatinine value was remarkably constant in all subjects on both diets and no difference was found in the two diets. The average creatinine value of all subjects was 1.4 mg. per 100 cc. of blood for both diets. On the other hand, creatine, while showing constant values on the same diet, was found in much higher concentration in the blood on the high protein diet, in spite of the fact that most of the protein of the diet was derived from milk and eggs. It would appear, therefore, that while the creatinine of the blood is maintained at a constant level in the same individual, independent of the protein of the diet, the creatine, on the other hand, rises and falls with the protein intake.

Attention should again be called to the very high non-protein nitrogen values on the high protein diet. The greater number of these figures are decidedly higher than the normal range allowed in most clinical laboratories.

3720

A Note on the Technic of Total Evisceration.

EDMUND ANDREWS.

From the Department of Surgery, University of Illinois College of Medicine.

For experiments of short duration (6 to 8 hours), total evisceration of a dog may be done successfully by the following simple method, which brings about nearly the same conditions as hepatectomy without the complicated series of operations leading up to the final stage.

Under very light ether anesthesia an incision is made from the xiphoid to a point half way from the umbilicus to the symphysis. The pelvic colon is clamped, ligated and cut, the ligature including enough mesentery to stop the inferior mesentery vessel. The distal end is not inverted. The entire intestinal mass is then delivered and a thin transparent mesentery can be cut, without clamping, to the level of the pancreas, where another set of vessels is met and clamped, the line of cleavage being close to the aorta. The choledochus, hepatic vessels and portal vein are next clamped en masse and the cardiac end of the stomach transfixed and ligated.

The entire mass of viscera can then be rapidly cut loose with one sweep of the knife. A heavy mass ligature is then thrown about the liver pedicle getting all the veins en masse. The three pedicles are ligated and the abdomen closed with a running stitch including all the layers. The procedure, with practice, may be carried out in 8 to 10 minutes, and the animal is left without liver, stomach, intestines, pancreas or spleen. In the few hours of life left, autolytic products of the ligated liver are not absorbed, as its capsule is untouched and the circulation entirely destroyed. The anesthetic is stopped after five minutes of operating, and glucose injections begun at once to counteract the rapidly ensuing hypoglycemia.

3721

Observations of Eviscerated Dogs.

EDMUND ANDREWS, WM. F. PETERSEN AND WM. BOIKAN.

From the Department of Surgery and Department of Pathology and Bacteriology, University of Illinois, College of Medicine.

During the course of previous experiments, we have reached the conclusion that the autonomic reorientation underlying the production of fever involves splanchnic stimulation (parasympathetic status) with peripheral inhibition (sympathetic status).[1]

During the time of chill the temperature of the muscle remains

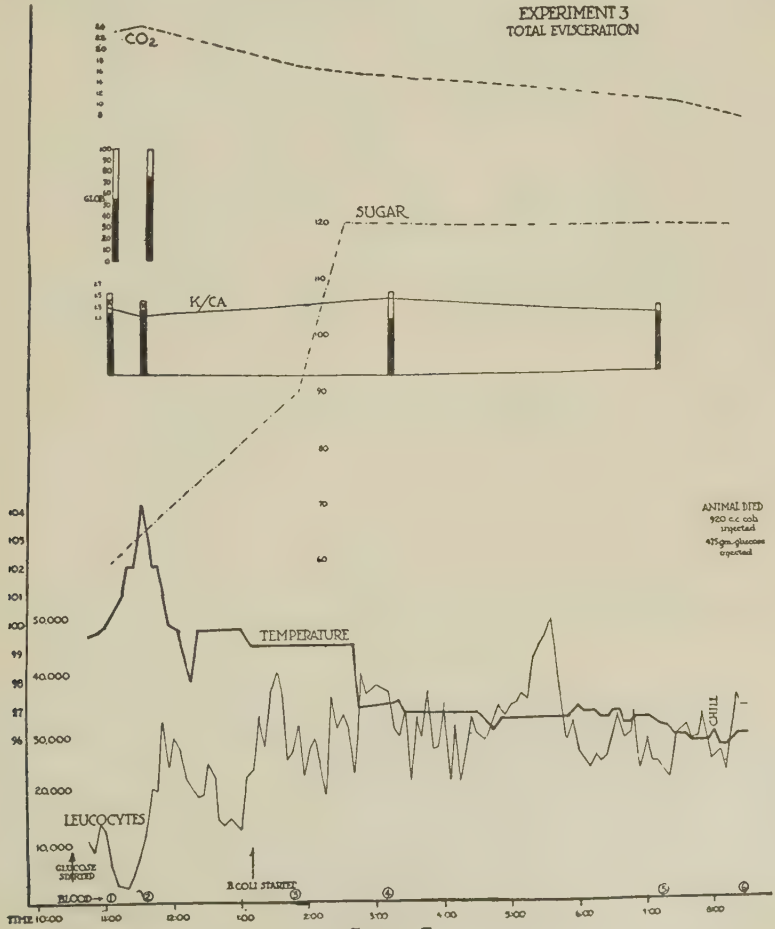

CHART I.

unaltered, or may be diminished at a time when the rectal temperature is rapidly increasing.[2] With this in mind we have carried out a number of observations on dogs which have been eviscerated according to the method described by Andrews.[3]

In our previous experiments we have made use of a continuous injection of a dilute suspension of *B. coli.* When so injected into the anesthetized animal, a short latent period is followed usually by the onset of a severe chill, which may last either continuously or intermittently for several hours.

In eviscerated animals which we have similarly injected (after the period of recovery following the operation) there was no evidence of chilling, nor was there an increase in rectal temperature. Of course these animals cannot be considered normal because they have a low CO_2 combining power and a very low K/Ca ratio.

We believe that the experiments offer additional evidence that the response of the splanchnic organs is of greatest importance in the production of fever. In eviscerated animals the effect of the bacterial injection on the liver is absent, the peripheral orientation (sympathetic) is not established, and heat loss is not retarded.

If the effect of the bacterial injection were directly on the so-called heat regulating center of the brain, the peripheral effect at least should be undisturbed.

The chart which we present is typical for experiments of this type.

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[1] Petersen, W. F., and Müller, E. F., *Klin. Wochens.*, 1926, v, 53-57.

[2] Petersen, W. F., and Müller, E. F., *Arch. Int. Med.*, in press.

[3] Andrews, E., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 108.

3722

The Lymph K/CA Ratio of Dogs During Continuous Intravenous Injection of B. Coli.

WM. F. PETERSEN, WM. BOIKAN AND E. F. MÜLLER.

From the Department of Pathology and Bacteriology, University of Illinois, College of Medicine.

In dogs with thoracic incanulation, prepared under local anesthesia, the continuous intravenous injection of dilute *B. coli* suspension, results in a series of chemical changes in the lymph blood, which can be correlated to the clinical picture.

In the normal animal, a prompt lowering of the K/Ca ratio takes place, followed by a series of fluctuations until a time of maximum injury occurs, when a ratio as low as 0.8 may be reached. The primary drop in the ratio (due to a slight increase in the calcium and a considerable decrease in the potassium) is evidently associated with a primary stimulation of the splanchnic organs. The cellular effort to reestablish the normal equilibrium probably accounts for the fluctuations in the ratio after the primary drop.

During the periods of low K/Ca ratio, an increase in the lymph volume and lymph concentration give evidence of a coincident increase in permeability of capillaries and tissues.

The CO_2 combining power takes a progressive course downward without marked fluctuations of the curve.

The period of maximum injury is usually followed by an antimortem increase in the ratio, due probably to the liberation of potassium from the red blood corpuscles. At this time a most marked gastro-intestinal symptomatology usually occurs (vomiting and diarrhea).

Animals in whom a low K/Ca ratio is present before the injection is started, have an initial response that is paradoxical, *i. e.,* the K/Ca ratio increases primarily. The complete details of the experiments will be published in the *Archives of Internal Medicine* and the *Journal of Infectious Diseases.*

3723

Skin Reactions with B. Typhosus Filtrate.

KATHARINE M. HOWELL AND MARION CORRIGAN.

From Kuppenheimer Fund and Otto Baer Fund for Clinical Research of the Michael Reese Hospital and the Nelson Morris Institute for Medical Research, Chicago.

For several years we have been studying skin reactions produced by a variety of bacterial filtrates in healthy persons, and in patients with different diseases. The recent typhoid epidemic in Montreal seemed to offer an unusual opportunity for further observations on the result of skin reactions with typhoid filtrates in typhoid fever patients. Through the courtesy of Dr. Ralph Lynch, of the Montreal General Hospital, observations were made on skin reactions of 22 typhoid patients in different stages of the disease. Intracutaneous tests with typhoid filtrates were also observed in other groups; for example, those who had no history of typhoid fever or of antityphoid vaccination, vaccinated persons, patients with other diseases, and 4 typhoid patients in Chicago.

After preliminary tests, the following technic was adopted: The typhoid bacillus employed in the test was the Rawling's strain. The medium used for the bacterial culture was a sugar-free, beef infusion broth 1% peptone, pH 7.4, to which was added 0.1% dextrose; the inoculated broth was incubated for 72 hours; then filtered through a Berkefeld filter and cultured for sterility. One :100 dilution, and in most instances 1 :500 dilution in 0.1 cc. amount was injected intradermally in skin of forearm. Uninoculated broth, incubated and filtered in similar manner to the inoculated broth was used for control skin tests. Positive skin reactions appeared within 18 to 24 hours and persisted for at least 48 hours. A strongly positive skin reaction had an elevated, dark red, indurated center at least 2 cm. in diameter, frequently surrounded by a less definite halo of 2 to 3 cm. The severe reactions lasted from 48 hours to a week, then became pigmented, and frequently there was a slight desquamation. In only 2 instances were there systemic reactions.

Six different groups of people were tested with the typhoid filtrate. The first group comprised 12 healthy adults who gave no history of typhoid fever or antityphoid vaccination. One person only reacted positively to the typhoid filtrate. The second group contained 11 persons who had received one or more immunizing courses of typhoid vaccine, 1 to 10 years prior to the skin tests. All reacted positively. The third group consisted of 4 persons who gave a history of typhoid fever, 2 years, 20 years, 23 years, and 30 years respectively before the skin tests were made. Three had strongly positive reactions, and one a weakly positive reaction.

The fourth group tested consisted of 26 patients in the course of typhoid fever, 22 of whom had positive typhoid blood cultures or positive Widals or both. Four of the typhoid cases occurred in Chicago and 22 in Montreal. Dr. Lynch injected these typhoid patients of the Montreal General Hospital with our typhoid filtrate and broth control in 1:100 and 1:500 dilution in 0.1 cc. amount. The results as shown by his description correspond to those obtained by us in persons reacting positively. In this series of 26 patients, 24 positive skin reactions occurred and 2 negative reactions. The two patients reacting negatively had both positive typhoid blood cultures and positive Widals. No correlation could be found between the severity of the skin reaction and the day of disease upon which the skin tests were made.

A fifth group of 88 adults with diseases other than typhoid fever were tested with the typhoid filtrate; 42 of these skin reactions were negative; 27 weakly positive and 19 strongly positive. A sixth group of 47 children, ranging in age from 3 to 15 years, received the intradermal tests with *B. typhosus* filtrate. Thirty-six of the children were in a cardiac camp and were not ill at the time the tests were made. The other 11 children were in a children's hospital ward and were convalescing from a variety of diseases. So far as could be determined, none had had typhoid fever or antityphoid vaccination. Nine positive reactions occurred in this group, but only two persisted 48 hours. One occurred in a boy 13 years old convalescing from an acute poliomyelitis, and the other in a boy of 13 who had a history of repeated attacks of acute rheumatic fever.

The skin reactions with the filtrate of *B. typhosus* suggest that the reaction is allergic in nature. A positive reaction apparently indicates immunity to typhoid fever, since those immunized against typhoid fever, those with a history of typhoid fever, and those in the course of typhoid fever tend to react positively in most instances, while only 40% of other adults and 4% of children react positively. The results indicate that preliminary skin tests might determine whether or not artificial immunization against typhoid fever was necessary in a given individual.

3724

Contraction and Evacuation of Gall-Bladder Caused by Highly Purified "Secretin" Preparation.

A. C. IVY AND ERIC OLDBERG.

From the Division of Physiology and Pharmacology, Northwestern University Medical School.

The numerous reports in the literature showing the marked efficacy of fats, egg yolk and protein in emptying the gall-bladder convinced us that the effect of "secretin" on the evacuation and motor activity of the gall-bladder should be studied, since it is well known that these substances stimulate the pancreas, and that "secretin" (very impure solutions) promotes the formation of bile.

For some time we (Kloster, Ivy and Lueth) have been working on the purification of "secretin". Starting with the "new secretin" of Weaver, Luckhardt, and Koch,[1] we prepared solutions of such purity that from 1 to 3 mg. of dried material excites pancreatic secretion in 10 to 20 kilo dogs. These purified solutions of "secretin" have been used in this study. They are vaso-dilatin free, and have no objective deliterious effects when injected intravenously in anesthetized or unanesthetized animals.

One series of experiments were performed as follows: A cat was barbitalized, the pancreatic duct was cannulated, and a cannula was placed into the gall-bladder through a small opening in the fundus. On the injection of the purified "secretin" (2 mg.) the intra-gall-bladder pressure rises after a very brief latent period, sometimes before the needle is withdrawn and always before the pancreatic juice and bile begins to flow. Normal saline controls proved negative. The pressure begins to decline in from ½ to 1 hour after the injection.

To avoid the possibility that the increase in gall-bladder pressure was not due to an inflow of bile, we tied the cystic duct or cannulated the bile duct. In the experiments in which the cystic duct was tied, the gall-bladder pressure was increased, but not to the extent of the first series of experiments. This difference, we believe, was due to some interference with the blood supply of the gall-bladder, which is very difficult to avoid in the cat on ligating or clamping the cystic duct. In the experiments in which the bile duct was cannulated before it entered the duodenal wall, the gall-bladder pressure was raised as much as in the first series of experiments.

When cats are prepared as above, the injection of 1 mg. of atropine sulphate does not have any effect on the action of the "secretin" preparation on motor activity of the gall-bladder.

In another series of experiments we filled the gall-bladder of the dog with lipiodol (iodized oil), using anesthesia and aseptic technic. From 12 to 24 hours later, the dog trained to lie quietly on the table, one dose of "secretin" was injected intravenously every 10 minutes for 1 hour, and roentgenograms were made two minutes after each injection. These injections practically emptied the gall-bladder in the course of an hour in some dogs; in others from one-third to one-half of the iodized oil was evacuated. In some of these dogs changes in the contour of the gall-bladder could be seen fluoroscopically. The above phenomena were observed when the dose of "secretin" amounted to as little as 4 mg. of solid material.

In one dog, the gall-bladder was visualized by using the tetraiodophenolphthalein technique. An intravenous injection of "secretin" every 10 minutes for 1 hour caused the gall-bladder shadow to decrease approximately ¼ in size. In 2 dogs the hepatic ducts were tied and the common bile duct ligated, the cannula being connected to the outside by means of a rubber tube which was clamped. Twenty-four hours after the operation, the animal lying quietly, the rubber tube was connected to a glass tube serving as a manometer, and a recording tambour. One dog's dose of "secretin" was injected every 4 or 5 minutes for 20 minutes. A steplike rise in the intra-gall-bladder pressure resulted; the pressure was raised in one instance from 180 mm. of bile pressure to 240 mm. in about 30 minutes, and maintained for a little more than ½ an hour, after which it started to decline. In barbitalized dogs, or dogs under light anesthesia, with the cystic duct clamped and the fundus of the gall-bladder cannulated, an injection of a "secretin" preparation causes a rise in intra-gall-bladder pressure.

These observations establish that a highly purified dilatin-free extract of intestinal mucosa on intravenous injection causes the gall-bladder to contract, *i. e.,* to increase its postural tone, and to evacuate. The active principle is either "secretin", or some substance closely associated with it. We have evidence to support the contention, that the duodenum plays some rôle in controlling the passage of bile into its lumen.

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[1] Weaver, M. M., Luckhardt, A. B., and Koch, F. C., *J. Am. Med. Assn.,* 1926, lxxxvii, 640.

Minnesota Branch.

Medical School, University of Minnesota, October 26, 1927.

3725

Antagonistic Action of Anesthetics and Alkali Metal Ions on Permeability of Cell.

J. F. MC CLENDON.

From the Marine Biological Laboratory, Woods Hole, and the Laboratory of Physiological Chemistry, University of Minnesota Medical School.

Some years ago in experiments on pike eggs, I showed that there is an antagonism between the action of Na ions which increased the permeability of the plasma membrane and anesthetics which prevented the increase in permeability. Anesthetics in large doses, however, increased the permeability and killed the eggs. It was not shown that anesthetics ever decreased the normal permeability. At Woods Hole, Mass., I recently performed similar experiments on Fundulus eggs. Isotonic solutions of $NaHCO_3$ and of the nitrates of Ca and Na were made and mixed so as to reproduce the cations in about the same concentrations as in sea water. In this solution or in distilled water practically no chlorides diffused out of the eggs. A micronephelometer was made by painting black the sides of the plungers of a (B. & L.) microcolorimeter, removing the mirror and illuminating it with parallel, horizontal rays. Ten mg. of KCl in a litre of H_2O was taken as the standard. One cc. of the standard + 1 drop of 10% $AgNO_3$ (acidified with HNO_3) was compared with 1 cc. of the unknown + 1 drop of $AgNO_3$ sol.* 100 eggs, washed until free from chlorides, were placed in 2 cc. of the solution. At the close of the experiment 1 cc. was removed for examination and the chloride content recorded in terms of the standard. Although sea water has a pH of about 8, the exosmosis of Cl^- was lowest at pH of 7 and less at pH 5 than at pH 8.

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* Control experiments showed that 2% alcohol did not interfere with the determination of chlorides.

Solution	Exosmosis of Cl- in 8 hrs.	16 hrs.
0.1 N $NaNO_3$	0.009	0.60
” ” containing 1% ethyl alcohol	0.006	0.28
” ” ” 2% ” ”	0.006	0.25
0.2 N $NaNO_3$		0.50
” ” ” 2% ” ”		0.27
Distilled water		0.18

From experiments not given in detail it appears that Na^+ and OH^- increase the permeability and Ca^{++} and H^+ inhibit their action. Alcohol has a similar effect to Ca^{++}.

3726

Iodine Content of Some Water Supplies in Goitrous Regions.

GERTRUDE HUMPHREY BECKWITH. (Introduced by J. F. McClendon.)

From the Laboratory of Physiological Chemistry, University of Minnesota.

The following determinations of iodine in drinking water of goitrous regions are uniformly low, except in the case of Lasalle, Ill. Lasalle has 2 water supplies. It is stated that there is less goiter among those persons drinking water from these drift wells than the other source of supply, which was not analyzed.

Source		Iodine Parts per billion
Litchfield, Illinois	Impounding Reservoir	0.17
Chicago	Lake Michigan	0.13
Peru, Illinois	Rock Wells (1300 ft.)	0.31
Peoria, Illinois	Drift Wells	0.15
Lasalle, Illinois	Drift Wells	18.00
E. St. Louis	Mississippi River	0.29
Champaign-Urbana	Drift Wells	1.01
Murphysboro, Illinois	Big Muddy River	0.30
Springfield, Illinois	Direct from Wells	0.50
Springfield, Illinois	Filtration Galleries	0.52
Duluth, Minnesota	Spring	0.014
Grantsville, Utah		1.33

3727

Reflex Time, Velocity of the Nerve Impulse and Duration of Muscular Contraction in the Tortoise.

M. CREMER, E. M. GREISHEIMER AND H. ROSENBERG.

From the Physiologische Institut der Tierärtzliche Hochschule, Berlin.

Stimulation by means of condensor shocks and induction shocks was found to be unsatisfactory in the study of the reflex time in non-curarized decapitated or decerebrated tortoises. The fall of a hammer from an electromagnet was used as a mechanical stimulus on the skin of the hind foot. A sharp point attached to the hammer served as a pain stimulus. The skin was covered by tin foil which by contact with a thin piece of copper, closed a circuit through a signal magnet as the hammer struck the tin foil, thus marking the time of stimulation. The leading-off electrodes were non-polarizable, of the zinc-zinc sulfate-sodium chloride type. An Edelmann string galvanometer was used.

A part of the shell was removed by a rotating machine-driven saw, so that the biceps cruris muscle could be used. The time between the stimulus and the electrical variation in this muscle was considered the reaction time. Nineteen determinations on 5 tortoises showed an average of 38.7 sigmas for reflex time. The reflex time was greater the longer the time after decapitation. In one tortoise the reflex time was 32.5 sigmas on the first day, 38.7 on the second, and 47.5 on the third. In another, it rose from 35.7 sigmas on the first to 49.5 on the second day. It seemed difficult to obtain crossed reflexes, but in a few satisfactory records the time for a crossed reflex was found to be 53 sigmas.

For a study of the velocity of the nerve impulse, the sciatic nerve and gastrocnemius muscle were used. The electrical stimulus was applied to the sciatic nerve through non-shielded electrodes, and the action current of the gastrocnemius was recorded. Single break induced shocks were used, and a signal magnet recorded the time of stimulation. The results were quite variable, but the average of 14 determinations was 15.1 meters per second.

The duration of contraction in the above records showed 33 sigmas for the biceps cruris and 24.4 sigmas for the gastrocnemius. All of the experiments were performed in March, in a laboratory in which the temperature averaged 20° C.

Since very little work has been done on the tortoise, it seemed advisable to call attention to the use of mechanical and pain stimuli, to the method of removing part of the shell in order to expose the nerves and muscles, and to the results so obtained.

New York Meeting.

Rockefeller Institute, November 16, 1927.

3728

Pasteurella Avisepticum* Infection of Poultry.

LESLIE T. WEBSTER, THOMAS P. HUGHES, IDA W. PRITCHETT AND FRED R. BEAUDETTE.

From the Laboratories of the Rockefeller Institute for Medical Research and the Poultry Department of the New Jersey Agricultural Experiment Station.

To extend our knowledge of epidemic diseases, we have undertaken a study of fowl cholera. Its microbic incitant, *Pasteurella aviseptica,* is similar to *B. lepisepticum,* the organism responsible for most of the snuffles and pneumonias of rabbits.[1] This latter disease being primarily an infection of the respiratory tract, suggested the possibility that *Pasteurella aviseptica* infects chickens by way of the air passages, leading to both the general manifestations commonly recognized as fowl cholera and the local involvements of the upper respiratory tract designated as "roup", "colds", etc. We have carried out experiments to test this theory, have made serial cultures of a nasal passage of White Leghorn fowls from 3 commercial flocks over a period of 6 months, and have carried out preliminary studies of the bacterial cultures obtained. This report summarizes the results.

A strain of *Pasteurella aviseptica,* obtained from Dr. Theobald Smith and designated Strain Pa, was administered intranasally to rabbits and mice. 0.01 cc. of an 18 hour rabbit's blood broth culture, dropped through the external nares over the nasal mucous membrane of rabbits, killed invariably within 24 hours. Septicaemia, petechial hemorrhages, and congestion of the lungs were found at autopsy.†

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* This organism is known as *Bact. avisepticum, Bacillus avisepticus, Bacillus bipolaris septicus* and bacillus of fowl cholera.

† This characteristic response of rabbits to the fowl cholera organism has been pointed out by many investigators since the time of Pasteur.

Similar procedures usually killed mice; a few survived, however, and carried the virulent organism for more than 6 weeks.

In the natural host, a somewhat different response was anticipated. Groups of chicks, 4 to 5 weeks old, uniform in weight, with no previous exposure to the infection, raised from similar stock and under uniform conditions, were given one drop of culture by way of the nasal cleft. Some died of acute cholera, others survived as carriers; some developed specific blood agglutinins; others seemed entirely refractory. Adult fowls, treated similarly, showed still an additional type of reaction. While some died of typical cholera, some displayed local lesions of the upper respiratory tract, clinically indistinguishable from "roup," "colds," diphtheria, and canker, others became "healthy" carriers. An occasional bird appeared entirely refractory.

No reaction followed the administration of the culture to chickens by mouth. Organisms were introduced into the esophagus by syringe, and by gelatin capsules. The latter method, while insuring against pharyngeal contamination, did no injury to the culture. The occasional fatalities which did occur were traceable to faulty technique. Stool cultures were persistently negative.[1] The results of these tests indicated that chickens were refractory to *Pasteurella aviseptica* given by the gastro-intestinal portal of entry, but susceptible to infection when the organisms were placed on the mucous membranes of the nasal clefts. Under these conditions, the same variations in type of clinical infection were noted as occur in rabbits when infected artificially or spontaneously with *B. lepisepticum.*[2] Hence we examined a number of commercial poultry flocks to discover whether the cholera organisms were carried in the droppings of "healthy" birds or in the nasal passages, and whether they were present in the local lesions, "roup," "colds," etc., as well as in the blood and lungs of typical cases of acute cholera.

Four distinct flocks were examined, 3 owned by commercial poultry men, and one maintained by the New Jersey Agricultural Experiment Station. The technique was essentially similar to that used in the investigation of rabbit snuffles-pneumonia.[1] The results to date are as follows: *Pasteurella aviseptica* was not found in the stools of "healthy" birds; it was present, however, in the nasal clefts of a considerable number. The presence of these carriers was definitely related to the amount of cholera and roup in a flock of young birds and in a flock of non-exposed birds. In a group of hens which had survived an acute epidemic of cholera, the carrier rate was very high (60%) and cases of local infection, roup, etc., were frequent.

A flock showing no carrier birds was free from cholera for the entire period of observation, although other pens on the same farm were infected. Autopsies on cholera and roup-"cold" cases from these flocks yielded organisms bacteriologically similar to those recovered from the nasal clefts of the "healthy" carrier birds.

The various strains from carriers, roup cases, and cholera cases are being studied further. For the most part they are similar, react the same culturally and serologically, and appear identical to the Strain Pa. When administered artificially to chickens, they lead to the same types of reaction as those described above.

We conclude, therefore, that *Pasteurella aviseptica* infection, like that of *B. levisepticum*, is primarily a respiratory disease, with the local manifestations commonly known as roup, "colds," etc., and the general pneumonia-septicaemia phase recognized as fowl cholera. The endemic focus of infection is believed to be the "healthy" nasal carrier or roup—"cold"—case, and the organisms from these various types of clinical disease are considered to be essentially similar.

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[1] Hertel (*Arb. a. d. Kais. Gesundheitsamt*, 1904, xx, H. 3) and Müller (*Monatschr. f. prakt. Tierheilk.*, Stuttgart, Enkes Verl., 1910, xxi, H. 9, No. 10) reported similar results.

[2] Webster, Leslie T., *J. Exp. Med.*, 1924-1927.

3729

Bone Marrow as an Organ.

FLORENCE R. SABIN AND CHARLES A. DOAN.

From the Laboratories of the Rockefeller Institute for Medical Research.

Before the mechanism of bone marrow as an organ could be analyzed, certain factors had to be clarified, among which are the structure of its vascular system and the maturation of blood cells. Chart 1 shows maturation as a constantly changing process with more than one variable. There are two critical points, (1) where the specific granules begin, and (2) where the last division of the myelocyte has taken place, giving a reduction to a common size and state of maturity, the leucocyte. The myelocytes are classified according to the number of granules, type A, having the first small clump of granules; type B, showing increasing numbers, and type C, the cell with the maximum number.

Supravital differential counts of blood and bone marrow of 5 normal and 2 abnormal rabbits are given in Tables I-III. Each survey included the epiphysis and shaft of each of the long bones and one rib. Table I shows that the normal rabbit's marrow contains about 70% granulocytes to 25% nucleated red cells. In early embryonic stages, only red cells are formed; during fetal stages, white cells are minimal; in the new-born rabbit the erythroid-myeloid ratio of the bone marrow is nearly the reverse of that of the adult.

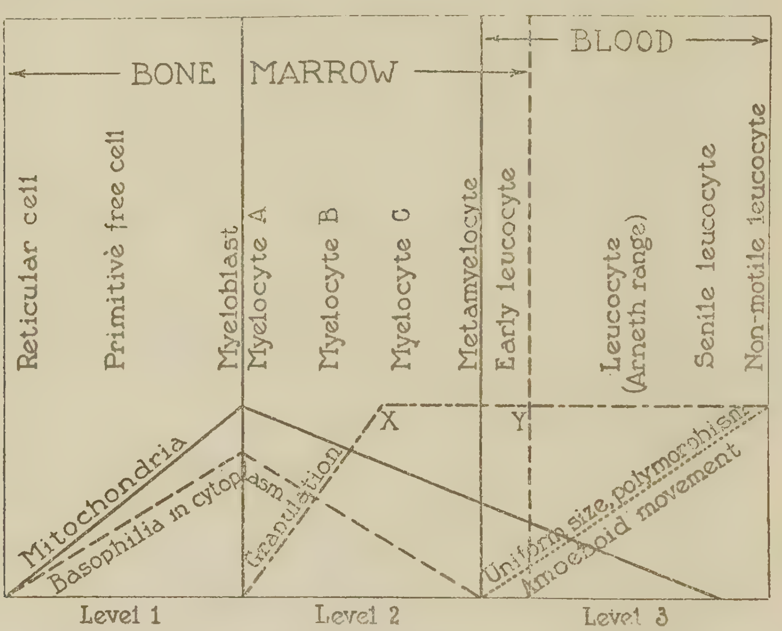

CHART 1. Diagram of the maturation of granulocytes.

The proportions of the stages of granulocytes are shown in Table II. The first 5 rabbits had normal blood and bone marrow, and in them the major supply of cells was of myelocytes C—(85%) with minimal numbers of types A and B and myeloblasts. The delivery of granulocytes from normal bone marrow is rhythmic, based on rhythmic and orderly division and maturation; the mechanism of delivery depends on a reciprocal relationship between myelocytes C and early leucocytes. The last 2 rabbits had a leucocytosis, pseudoeosinophiles 71% and 68%, with the normal at 44%. Corresponding to this change in the blood, the marrow had a characteristic pattern; in Rabbit 19 B myelocytes type B were 19.29%; in Rabbit 13 B, the type B were 48.59% and type A 2.87%. This is a shift toward the immature stages detected by cytoplasmic criteria, comparable to the Arneth "shift to the left" for the leucocytes.

The classification of myelocytes by the number of granules is a more sensitive index than their changing chemistry, for the term "promyelocyte", the cell with unripe granules, combines types A and B and probably part of type C into one group.

The pattern of the marrow for red cells (Table III) shows that the major supply is at the stage of the normoblast (69%) with smaller numbers of late erythroblasts and minimal numbers of early erythroblasts and megaloblasts. The delivery of red cells is through a reciprocal relationship between normoblasts and the reticulated red cells.

TABLE I.

Showing the erythroid-myeloid ratio in blood and bone marrow in rabbits. Supravital technique.

Rabbit No.	Peripheral blood.			Bone marrow.			Number of cells counted
	W.B.C.	Myeloid cells (Total No.	R. B. C.	Total myeloid	Total erythroid	All other cells	
1B	15,900	42% (6678)	6,360,000	72%	26%	2%	4800
2B	12,600	40% (5040)	6,130,000	71%	27%	2%	4200
3B	14,900	45% (6705)	5,800,000	71%	19%	10%	7500
6B	11,900	48% (5712)	6,230,000	71%	21%	8%	7300
7B	8,900	46% (4094)	5,230,000	68%	28%	4%	6500
13B	12,200	83% (10,120)	5,610,000	63%	27%	10%	4800
19B	14,800	71% (10,508)	6,050,000	59%	37%	4%	5900

TABLE II.

Showing the percentage of types of cells within the myeloid group in bone marrow in rabbits. Supravital technique.

Rabbit No.	P. M. N.	Neutrophilic myelocytes			Myelocytes		Myeloblasts	Myeloid cells counted
		C	B	A	Eosinophilic	Basophilic		
1B	3.15	92.10	1.84	0.11	2.06	0.74	0.	3,484
2B	7.34	79.38	8.35	0.43	2.37	1.57	0.56	2,988
3B	3.96	87.33	3.46	0.58	2.10	0.40	2.17	5,277
6B	1.93	90.87	3.16	0.36	1.22	0.84	1.62	5,215
7B	7.13	78.33	5.09	0.56	4.58	2.98	1.33	4,358
Average for five normal rabbits	4.66	85.72	4.36	0.41	2.45	1.29	1.11	21,322
19B	19.99	54.43	19.29	0.76	2.36	2.95	0.22	3,514
13B	8.78	32.27	48.59	2.87	3.60	2.97	0 92	3 027

TABLE III.

Showing the percentages and total numbers of all the cells of the bone marrow in rabbits. Supravital technique.

Rabbit No.	P. M. N.	Neutrophilic myelocytes			Myelocytes "C"		Myeloblasts	Primitive	Clasmatocytes	Megakaryocytes	Monocytes	Lymphocytes	Red blood cells			Total cells counted
		C	B	A	Eosino-philic	Baso-philic							Normo-blasts	Erythro-blasts	Megalo-blasts	
1B	2.28%	66.47	1.34	0.06	1.44	0.53	0	0	0.44	0.49	0.36	0.06	22.83	3.70	0	
	110	3209	65	4	70	26	0	0	22	24	18	3	1097	179	0	4827
2B	5.18%	55.93	5.87	0.30	1.67	1.08	0.40	1.25	0.28	0.47	0.18	0.07	20.78	6.44	0.04	
	220	2371	249	13	71	46	17	53	12	20	8	3	881	273	2	4239
3B	2.71%	61.30	2.43	0.40	1.47	0.29	1.51	9.91	0.23	0.26	0.05	0.09	15.15	4.05	0	
	209	4607	183	31	111	22	114	745	18	20	4	7	1139	305	0	7515
6B	1.39%	64.80	2.17	0.25	1.06	0.53	1.09	6.49	0.87	0.51	0.05	0	13.40	7.34	0	
	101	4739	159	19	78	39	80	475	64	38	4	0	980	537	0	7313
7B	4.85%	53.31	3.46	0.37	3.12	2.02	0.81	1.87	0.96	0.79	0.10	0.01	14.31	13.85	0.01	
	311	3414	222	24	200	130	57	120	62	51	7	1	917	887	1	6404
13B	5.47%	20.44	30.53	1.80	2.26	1.83	0.58	7.86	1.08	0.62	0.08	0.02	17.39	9.96	0	
	263	983	1468	87	109	89	28	378	52	30	4	1	836	479	0	4807
19B	11.77%	32.14	11.39	0.45	1.39	1.74	0.13	2.23	0.94	0.63	0.05	0	22.40	14.74	0	
	701	1913	678	27	83	104	8	133	56	38	3	0	1335	878	0	5957

The structure of bone marrow is adapted to the maintenance of an erythroid-myeloid ratio, the regulation of the proportion of the stages of maturation of both red and white strains, and the delivery of cells to the blood. The regulation of these factors is in part vascular and in part chemical. With red cells developing in collapsed capillaries, and white cells developing extravascularly near dilated sinuses, the mechanism that controls the proportion of collapsed to dilated sinuses regulates in part the erythroid-myeloid ratio. The chemical forces that act on the marrow cells, as distinct from those that act on the endothelium, are divided into two activities: chemotactic factors (C) that attract the cells into the blood, and growth-stimulating, or maturation (M) factors for both red and white strains. Certain chemotactic factors for the white cells are known—bacterial proteids and the physiological product, nucleic acid. A maturation factor for red cells has been discovered by Minot and Murphy[1, 2] in liver.

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[1] Minot, G. R., and Murphy, W. P., *J. Am. Med. Assn.*, 1926, lxxxvii, 470.

[2] Murphy, W. P., and Minot, G. R., *Boston Med. and Surg. J.*, 1926, clxxxxv, 410.

3730

Vitamin B Testing Revised.

WALTER H. EDDY.

From the Laboratory of Physiological Chemistry, Teachers' College, Columbia University.

The demonstration by Smith and Hendrick,[1] Goldberger and co-workers,[2] and others[3] in this country that what we have called vitamin B is composed of at least two factors, each with specific functions and properties, necessitates the revision of existing vitamin B tests. In England, Chick and Roscoe[4] have been able to confirm the contentions of Goldberger. They showed that wheat embryo is distinctly richer in antineuritic factor than in antipellagric factor, by utilizing autoclaved yeast as the source of antipellagric and a preparation of Peters to supply the antineuritic. The Peters' fraction was separated by Kinnersley and Peters[5] of Oxford University by controlling the selective adsorption of norite. During the past year Williams and Waterman of our laboratory have also succeeded in separating a yeast fraction which is antineuritic, but is apparently free of the factor (antipellagric) which is present in autoclaved yeast. Their separation was attained by controlling the selective adsorption of fuller's earth.[6]

In contrast to the alcoholic extract of corn meal used by Goldberger, Williams' preparation has supplied a testing fraction entirely devoid of antipellagric factor. To date we have been able to use this preparation in retesting two sources of vitamin B.

Last year we reported that ripe banana tested by the Sherman method must be supplied to rats on B-free, but otherwise adequate diet, in quantities of 8 to 10 gm. daily, to permit a growth gain of 20 gm. in 60 days. Using 5 mg. daily of the Williams' preparation as adequate source of antineuritic, we find that 2 gm. of banana daily supplies enough antipellagric factor to produce 20 gm. gain in 60 days, and that 6 gm. daily is nearly adequate for normal growth. By using autoclaved yeast as antipellagric, we find that at least 6 gm. of banana daily must be added to prevent growth decline. In brief, the banana is at least three times as rich in antipellagric factor as in antineuritic, though both are present.

Osborne and Mendel[7] reported that 1 gm. of dried fresh spinach would support normal rat growth when used as a sole source of vitamin B in an otherwise adequate diet. Kohman aand Eddy[8] confirmed this result in more recent tests. We found that rats would not eat enough of dried canned or cooked spinach to accomplish this effect but would eat it and grow normally if 1/3 of their total B requirement was met by dried yeast. At the time these tests were made Williams was unable to protect pigeons from polyneuritis with a dosage of 4.4 gm. dried canned spinach, and this observation was noted in our report at the time. At the time also we interpreted our yeast-spinach results as a matter of appetite stimulation rather than absence of the vitamin in the canned product.

We have recently repeated tests on cooked spinach with the aid of the Williams' preparations and autoclaved yeast. Home cooked, fresh spinach is about 98% water. However, when the rat's antineuritic requirement is met by 5 mg. daily of Williams' preparation, 2 gm. daily of the undried cooked spinach supplies enough antipellagric to produce nearly normal growth in test animals. This amount fails to supply enough of both factors to prevent growth decline, but when combined with 0.5 gm. daily of autoclaved yeast it will prevent such weight decline. Cooked fresh spinach, therefore, contains some antineuritic factor, but like the banana is richer in antipellagric than in antineuritic. The quantitative distribution of the two factors in dried fresh spinach, cooked and canned spinach, awaits further tests.

These two preliminary studies indicate the possibility of quantitative revision of our vitamin B tables, through use of the Peters' or the Williams' preparations. The study of Williams' preparation in bird tests has shown, however, that while antineuritic for these animals and capable of checking weight decline, it is not able to restore them to normal weight. Autoclaved yeast also fails to provide the restorative for birds though effective in rats. Finally yeast or whole grains prove both antineuritic and restorative of weight for pigeons. As Williams and Waterman have pointed out, these factors appear to indicate that yeast and whole grains contain a B factor which is not antineuritic, not present in autoclaved yeast and necessary to bird weight control. Our tests on Peters' fraction are not yet sufficiently advanced to state whether his fraction is like Williams' or different, but in the only two observations reported by Peters it failed to prevent weight decline in pigeons.

The doubts raised by the above tests as to whether vitamin B is dual or still more multiple in nature, are obvious, and suggest further study of the vitamin B fractionation before attempting extensive revision of the existing tables. Tools are, however, now available for assaying at least two of these factors with the rat as test animal.

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[1] Smith and Hendrick, Public Health Report, U. S. P. H., 1926, xli, 201.

[2] Goldberger and coworkers, Public Health Reports, U. S. P. H., 1926, xli, 297, and U. S. P. H., 1926, xli, 1025.

[3] Salmon, *J. Biol Chem.*, 1927, lxxiii, 483; Hauge and Carrick *J. Biol. Chem.*, 1926, lxix, 403.

[4] Chick and Roscoe, *Biochem. J.*, 1927, xxi, 698.

[5] Kinnersley and Peters, *Biochem. J.*, 1925, xix, 8-20, and personal communication of later date.

[6] Williams and Waterman, PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 63.

[7] Osborne and Mendel, *J. Biol. Chem.*, 1919, xxxvii, 187.

[8] Eddy, Kohman and Carlsson, *J. Indust. and Eng. Chem.*, 1925, xvii, 69.

3731

Multiple Partition Coefficients of Penetration.

MARIAN IRWIN.

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Overton's lipoid theory[1] which states that only dyes soluble in lipoid can penetrate into living cells, is inadequate in certain respects.[2] This is due in part to the fact that he considered only one partition coefficient, that of the dye between the external solution and the lipoid layer surrounding the living cell. Since it is chiefly cytoplasmic inclusions, granules, fat globules, vacuoles, etc., which are stained rather than the cytoplasm proper, it is necessary to consider several partition coefficients, such as: (1) That of the dye between the non-aqueous layer surrounding the cytoplasm and the external solution. (2) That of the dye between the same non-aqueous layer and the aqueous cytoplasm. (3) That of the dye between the aqueous cytoplasm and the inner non-aqueous inclusions (granules, or fat globules) or the non-aqueous layer surrounding the vacuole. (4) That of the dye between the inner non-aqueous layer of the vacuole and the aqueous vacuolar sap.

Experiments from this point of view have been made on *Nitella flexilis* and *Valonia macrophysa*. The results are presented here with *Valonia*, the cells of which consist of an outer cell wall, an inner protoplasmic layer surrounding a relatively large central vacuole filled with sap, at about pH 5.8 and containing about 0.6 M halides.

We may assume[3] that the dye diffuses through the protoplasm into the vacuole in succession through an outer non-aqueous layer, a middle aqueous layer, an inner non-aqueous layer (surrounding the vacuole), and then into the vacuolar sap.

By applying these ideas experimentally we can predict the behavior of the dyes in penetrating *Valonia*. The dye in sea water at pH 9.5 or at pH 5.5 is shaken with chloroform so as to represent the two non-aqueous layers. Then the chloroform is removed and shaken with freshly collected *Valonia* sap. We should then expect that the dye, which penetrates the vacuole most readily, is that which is most readily absorbed by chloroform from sea water, and is most readily given up by chloroform to the sap. This is found to be the case. The penetration of dye into the vacuole of living cells was measured by placing the living cells in about 0.03% dye dissolved in sea water at pH 9.5 and at pH 5.5 for 1 to 3 hours. The amount of dye found in the vacuole was determined in the manner already described.[4]

The experiments show the following results: (1) Trypan blue, trypan red, and Bordeaux red are not absorbed by chloroform from sea water, and they do not readily* enter the vacuole (*viz.*, no dye penetrates in 3 hours). (2) Acid fuchsin and basic fuchsin are very slightly absorbed by chloroform from sea water, and they are readily absorbed from the chloroform by the sap. They do not readily enter the vacuole (only a trace of basic fuchsin is found from pH 9.5 after 3 hours). (3) Crystal violet and methyl violet are readily absorbed by chloroform from sea water and are not readily given up to the sap. They do not readily enter the vacuole. (4a) From methylene blue dissolved in sea water at pH 9.5, azure B is chiefly absorbed by chloroform. Azure B is also chiefly absorbed by sap from this chloroform. It is chiefly azure B that penetrates the vacuole from methylene blue in sea water at pH 9.5. (b) Practically no thionin is absorbed by chloroform from sea water. A violet-pink dye is found in chloroform, and is absorbed by the sap. The dye which is found in the vacuole is also violet-pink, and not chiefly thionin which is violet. (c) From methyl green in sea water, chiefly methyl violet is absorbed by chloroform, but only methyl green is given up to the sap. Mostly methyl green and not methyl violet penetrates the vacuole. (5) Brilliant cresyl blue, azure B, toluidine blue, new methylene blue, methylene green, are more readily absorbed by chloroform from sea water at pH 9.5 than at pH 5.5. These dyes penetrate the vacuole more readily from sea water at pH 9.5 than at pH 5.5.

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* The phrase "does not readily enter" is used even in the case when no dye enters during the experiment because it might be found to enter with a much longer exposure.

There are, of course, certain factors which must be considered. If a dye enters more readily in one form than in the other (*e. g.*, free base[5] as contrasted with the salt of a basic dye), the rate of penetration and degree of accumulation will depend in part on the ratio of one form of dye to the other.

The penetration, accumulation, and exit of the dye may also depend on compounds formed between the dye and the various substances in the cell.

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[1] Overton, E., *Jahrb. wissensch. Bot.*, 1900, xxxiv, 669.

[2] For reviews of the literature see Robertson, T. B., *J. Biol. Chem.*, 1908, iv, 1, and Höber, R., Physikalische Chemie der Zelle und der Gewebe, 6th ed., Leipzig, 1926.

[3] For a diagram of the cell see Irwin, M., *J. Gen. Physiol.*, 1926-27, ix, 75.

[4] Irwin, M., *J. Gen. Physiol.*, 1926-27, x, 927.

[5] Irwin, M., *J. Gen. Physiol.*, 1925-26, ix, 561.

3732

Occurrence of Atypical Glomeruli in the Kidney of Opossum (Didelphys Virginiana)*

WM. DE B. MAC NIDER.

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The histological structure of the glomerulus in the dog, cat, rabbit, and guinea pig, used in various studies of renal function, is, in general, similar. The glomeruli vary in size and number in such animals, and the degree of lobulation of the capillary tufts shows some difference in the various species, yet the finer structure of these bodies remains constant. The epithelium which lines Bowman's capsule consists of a single layer of flattened, non-specialized cells and the reflection of these cells over and between the capillary loops of the glomeruli is made up of an even flatter type of cell.

In 1924 a study was commenced of the histology of the kidney in certain older animals to ascertain if there was any difference in structure in the vascular and tubular units in such ancestral species which might be indicative of a difference in the function of these structures as compared with similar units in more recent species, now used for experimental purposes. The first animal studied was the marsupial, *Didelphys virginiana.*

Twenty-seven opossum have been used in this study. The animals were placed in large types of rabbit cages and the urine collected. The urine from all of the animals was normal. It was free from albumin, glucose and diacetic acid. The microscopic studies showed an absence of casts. Various types of epithelial cells with occasional red and white blood cells were present. After such initial observations the animals were killed by a stroke behind the head, and the kidneys immediately removed for fixation. The kidneys were sectioned through their long axes, and tissue for fixation removed from each lateral half. The fixatives employed were 95% alcohol, Zenker's fluid, 10% formalin, and a solution of corrosive-acetic. Both paraffin and celloidin sections were made and stained with eosin and haematoxylin and with eosin and methylene blue.

The histological study of the kidneys of 19 of the 27 opossum showed glomerular structures similar to those in higher laboratory animals and man. The tubular epithelium and especially that of the convoluted tubules appears to be less specialized than epithelium in the same location in more recent species. Very frequently the convo-

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* Aided by a grant from the Ella Sachs Plotz Foundation.

luted tubule cells fail to show a clear-cut differentiation. In such areas the nuclei have not a regular arrangement, so that a syncytial-like appearance is produced. The cell cytoplasm is not striated. The epithelium of the loops of Henle and the straight tubules have the usual histological structure. The cell boundaries are definite and the nuclei have a regular arrangement.

In 8 of the 27 opossum kidneys, in addition to the occurrence of a predominance of normal glomeruli, an atypical type of glomerulus was found. These atypical structures vary in size, as is the case with the normal glomeruli. The capillary endothelium and the lobulation of the tufts show no departure from the normal. The structure in these glomeruli which makes them atypical and of interest, is the epithelium which lines Bowman's capsule. In this location in place of the usual flattened, non-specialized type of cell, there are high columnar cells showing a definite demarcation one from the other and containing large, regularly arranged, hyperchromatic nuclei. This atypical epithelium has the cytological appearance of marked specialization. The height of the cells was found to vary in the different glomeruli, and in the same structures these cells showed some evidence of a transition from the high columnar type to a more flattened variety of cell. In such transition there was found no approach to the usual flattened epithelium which lines Bowman's membrane in the normal glomerulus.

To ascertain the numerical relationship of the atypical glomeruli to the normal glomeruli, the total number of both types of glomeruli were counted in 50 sections. Each section represents a cross-section taken from tissue obtained at the mid-portion of the lateral half of the kidney. The average for both types of glomeruli in such sections is 243. Of this number 84 are atypical and 159 are normal glomeruli. The tubular epithelium in the kidneys of the 7 opossum with atypical glomeruli has in general the same cytological appearance as the 19 normal animals. The convoluted tubule epithelium in many of the tubules has a syncytial appearance with no systematic arrangement of the nuclei. There is a lack of cell differentiation. (See Fig. 1.)

Insufficient data have been accumulated and no functional studies have been made which at the present time will permit a discussion of the origin or meaning of the atypical glomeruli in the kidney of the opossum. The question arises whether or not in the kidney of some of the marsupials and in species of animals of an even more remote biological position, the glomerulus had a more specialized structure, and a more specialized function than it now has in the higher laboratory animals and in man.

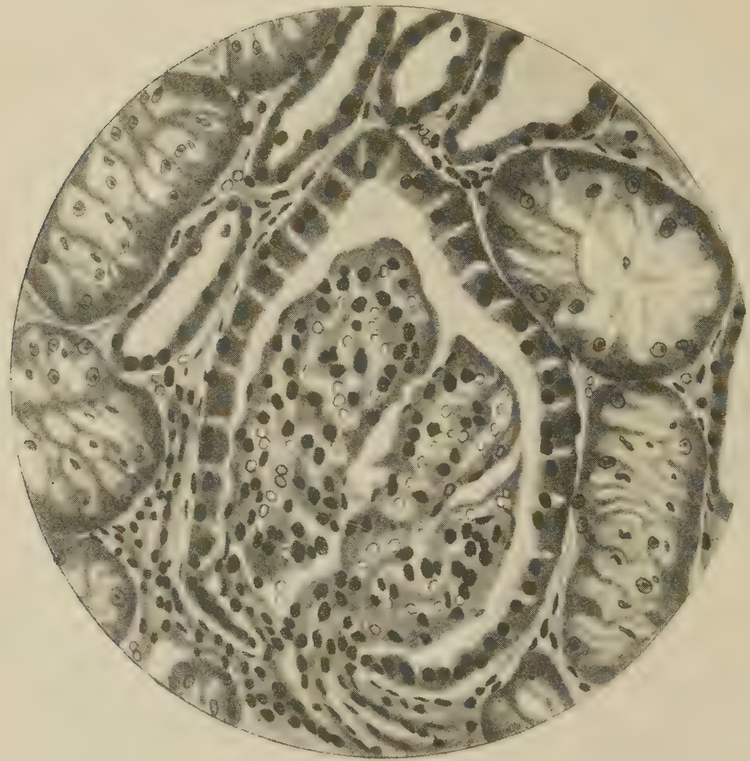

FIG. 1.

Photograph without enlargement of camera lucida drawing, Leitz, Oc. 2, Obj. 6, from the kidney of Opossum 4. In the center is large glomerulus. Capillary tuft shows a definite lobulation. The epithelial layer of cells reflected over the capillaries is of the usual flattened type. The epithelium lining Bowman's membrane is made up of a high columnar type of cell atypical for this location. The cells are clear-cut in their differentiation. The nuclei of these cells show a regular arrangement and are hyperchromatic. The convoluted tubule epithelium shows a lack of specialization in structure. In certain of the tubules a syncytial appearance is obtained. The epithelium of the loops of Henle is normal.

Similar investigations are now in progress with the kidneys of the Duck-Bill, *Ornithorhynchus anatinus* (*Paradoxus* Blumenb.), the Spiny Ant-eater, *Echidna aculeata,* and an Australian lizzard, *Sphenodon* or *Hatteria punctatum.*

I am indebted to Professor Henry A. Christian of the Harvard Medical School for securing this tissue for me from the National Museum of Australian Zoology.

3733

New Series of Reagents Useful in Fractionation of Biologically Active Material.

CASIMIR FUNK.

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Aside from the classical procedure of Kossel and Kutscher for the fractionation of nitrogenous bases, we possess no methods which permit a systematic biochemical analysis of nitrogenous substances occurring in nature. The Kossel procedure, although extremely useful for the fractionation of stable substances, interferes very often with the activity of relatively unstable substances, such as vitamines, hormones and enzymes. Various aromatic nitro compounds have been extensively used in biochemistry, such as picric, picrolonic and flavianic acids. These reagents often yield characteristic, slightly soluble compounds with relatively pure substances, but often give little or nothing when we deal with complicated mixtures. The idea occurred to me that by using the salts of these acids a series of more specific reagents could be obtained. This expectation was realized. Where the free acid fails to affect a notable separation of active material, this was accomplished by using a salt of a particular acid, or a series of salts, one after the other. The salts used so far were barium, lead, silver and mercury salts, metals which can be removed from the solution without difficulty. In some cases sodium salts were used with success. The method was used either systematically, starting with picric acid and then using the various salts, or one particular salt was used exclusively. It is hoped that by introducing other salts, or salts of other acids, a systematic biochemical analysis of unknown substances can be effected.

Using this new method, vitamine B was separated quantitatively from yeast as well as from rice polishings, but curiously enough, not by the use of one single salt. A combination of Ag- and Hg-picrates was necessary to attain this end, which corroborates my view perhaps as to the complex nature of this substance. Contrary to the results of Jansen and Donath,[1] no trace of anti-beriberi vitamine could be precipitated from the decomposed picrates by means of platinum chloride in absolute alcohol. This substantiates my earlier findings in 1911. It also seems that vitamine C can be precipitated by a similar procedure, but quantitative data are not yet available.

The method has been applied also to the fractionation of hormones. With insulin no marked success has yet been attained. With pituitrin, on the contrary, the oxytoxic activity could be ascribed to a simultaneous action of several substances[2] which, taken separately, showed little action on the uterus. Finally, by the use of sodium picrate in a very concentrated solution, pepsin could be divided into inactive ferment (propepsin) and the kinase. Detailed reports will be published.

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[1] Jansen and Donath, *Gen. Tijdschr. v. Ned-Indie,* 1926, lxvi, 6.

[2] Reported at the Intern. Congress of Physiol., Stockholm, 1926. Comp. Report of Oliver Kamm at the meeting of Amer. Chem. Soc. in Detroit, 1927.

3734

Determination of Blood pH Without Transferring the Sample.

IRVINE MC QUARRIE.

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The simple procedure described here has been devised to obviate the necessity of transferring the blood and of using mineral oil, both of which tend to complicate the technique for determining the pH colorimetrically. The only special apparatus* required for this consists of a sampling tube identical in shape and size (10x1.5 cm.) with the tubes used to contain the standard solutions. It is provided with a double-holed, hollow, glass stopper, which is ground-in and which has sealed to it a small, two-way stop-cock with two holes. (Fig. 1.) The apparatus is made of clear Pyrex or Nonsol glass and connections are made with special rubber tubing, which will not cause excessive film formation on the mercury. Before use all apparatus, including the mercury, is cleaned and tested for neutrality in a manner similar to that outlined by Austin, Cullen, *et al.*[1]

The general method followed is essentially that of Cullen[2] as modified by Hastings and Sendroy.[3] Whole blood is used instead of plasma in accordance with the method of Hawkins.[4] The necessary correction factor for converting the colorimetric readings to the true pH value is found by the method proposed by Austin, Stadie and Robinson.[5]

When a determination is to be made, the apparatus is assembled as shown in the accompanying figure. Mercury is run from the

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* May be obtained from Technical Glass Apparatus Co., 42 Galusha Street, Rochester, New York.

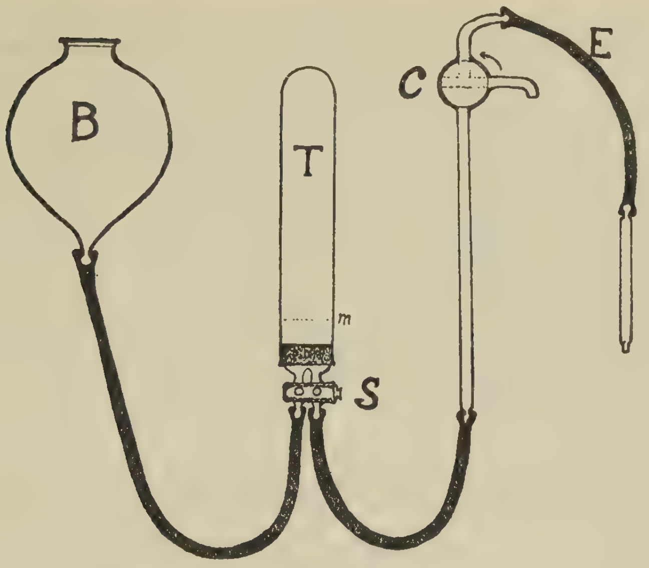

FIG. 1.
Apparatus as Assembled for Taking Blood Sample.

bulb B into the sampling tube and connecting tubes until these are completely filled to the three-way inlet stop-cock C, thereby excluding all air. The sampling tube held by a clamp attached to the stop-cock S is then inverted. With the stop-cocks S and C open to make direct communication between T and E, the latter is immersed in a small flask of indicator-saline solution, which has been adjusted previously to a pH of approximately 7.40, and the air in E is displaced with mercury by elevating B. The latter is then lowered until the necessary amount of diluting fluid has been drawn into T. In order to make the required 20-fold dilution of the plasma, the amount of blood drawn into the sampling tube must be such that its plasma volume is 1/20 (or slightly less) of the capacity of the tube. In preparation for taking the blood, therefore, a volume of mercury equal to that of the sample to be taken must be left in the inverted sampling tube under the diluting fluid (level m).

With the sampling tube still in place, the color of its contents through an additional tube filled with distilled water is compared with the 7.40 bicolorimetric standard. If these do not match, the fluid is run back into the flask and 0.01 N NaOH or HCl, as indicated, is added until the match is satisfactory. The connecting tube is refilled with mercury as far as stop-cock C by elevating bulb B, after which C is closed.

With the diluting fluid thus properly adjusted, vena puncture is made under aseptic conditions. After all air and fluid have been forced out of E by the blood, the mercury bulb is lowered and C is opened long enough to allow the mercury in the sampling tube to be replaced by blood. The stop-cock S is then closed and T is inverted to mix its contents. With the rubber connecting tubes detached, the sampling tube is centrifuged until its supernatant fluid is entirely clear. This is then placed with the standard tubes in a water bath at 38.5° C. for 10 minutes before it is matched with these in the comparator block.

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[1] Austin, J. H., Cullen, G. E., Hastings, A. B., McLean, F. C., Peters, J. P., and Van Slyke, D. D., *J. Biol. Chem.*, 1922, liv, 121.

[2] Cullen, G. E., *J. Biol. Chem.*, 1922, lii, 501.

[3] Hastings, A. B., and Sendroy, J., Jr., *J. Biol. Chem.*, 1924, lxi, 695.

[4] Hawkins, J. A., *J. Biol. Chem.*, 1923, lvii, 493.

[5] Austin, J. H., Stadie, W. C., and Robinson, H. W., *J. Biol. Chem.*, 1925, lxvi, 505.

3735

Relation of Calcium and Phosphorus in Blood of Parathyroidectomized Dogs.

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Changes in the blood calcium and phosphorus in dogs were investigated, both before and after complete thyro-parathyroidectomy. While it was found that tetany occurred at varying levels of calcium concentration, there was always a definite mathematical relation between the concentrations of the two elements when symptoms first appeared. In normal animals the Ca:P ratio ranged between 1.5 and 2.0. After operation there was progressive reduction in this ratio until the first attack of tetany, when it was always approximately 1.0. Occasionally, there was an increase after spontaneous recovery from an attack, but usually there was a progressive decrease with each successive attack until death occurred. Treatment by any of the established methods restored the ratio, but symptoms always disappeared long before there was any extensive readjustment of the ratio. The following table illustrates the relationship:

TABLE I.

No.	Ca mg. per 100 cc.	P mg. per 100 cc.	Ca:P	
21	11.65	5.10	2.28	24 hours after treatment, no tetany.
	6.22	6.78	0.917	Tetany and spasticity.
36	11.30	4.44	2.545	Day before operation.
	5.60	6.36	0.880	5 days after op. First attack.
	9.60	4.75	2.022	15 days after op. 24 hrs. after treatment. No tetany.
	5.44	5.87	0.926	17 days after op. Severe tetany.
43	11.47	5.71	2+	2nd day before op.
	8.87	5.01	1.5	2nd day after op. No tetany.
	5.88	8.20	0.717	3rd day after op. Mild tet. 2nd attack.
	5.19	9.43	0.551	7th day after op. Severe tetany.

3736

The Influence of Amytal Upon Blood Sugar Content.

FRANK P. UNDERHILL AND DOUGLAS H. SPRUNT.

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The statement of Page[1] that during amytal narcosis no perceptible changes in blood sugar content occur was so suggestive for new possibilities in the study of carbohydrate metabolism as to warrant a repetition of the experiments of Page. In our work, the rabbit

TABLE I.
The Influence of Amytal upon Blood Sugar Content in the Rabbit.

Rabbit Number	1	3	4	6
Weight in grams	2000	2200	2200	2100
Method of introduction of drug	Per os	Subcutaneous	Intraperitoneal	Intraperitoneal
Mg. per kilo	80	80	50	100
Blood sugar content in mg. per 100 cc. of blood.				
Before injection of drug	122	100	107	140
30 minutes later	138	130	131	140
45 minutes later	148	145	185	198
1½ hours later	138	185	155	267
2 hours later	—	—	—	336
3 hours later	—	286	160	—
4 hours later	—	—	—	285
5 hours later	123	221	—	Died

served as the experimental animal. In a series of 10 experiments 8 gave evidence of a distinct hyperglycemia which usually appears within an hour, or somewhat later, and may last for several hours. The hyperglycemia is induced by doses ranging from 50 to 100 mg. per kilo of amytal whether given by mouth, subcutaneously or intraperitoneally. Since the completion of our work Weiss[2] has shown that amytal produces hyperglycemia also in the cat and dog.

Illustrative examples of the experiments are given in Table I.

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[1] Page, I. H., *J. Lab. and Clin. Med.*, 1925, ix, 194.

[2] Weiss, S., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 363.

3737

Concentration of Arsenic in Blood Subsequent to Serial Administration of Neoarsphenamine.

FRANK P. UNDERHILL AND MAX HORN.

From the Department of Pharmacology and Toxicology, Yale University.

A number of investigations* have shown that when arsphenamine and neoarsphenamine are intravenously introduced, the arsenicals rapidly disappear from the blood stream, part apparently going to immediate excretion, and part to storage in the tissues. Usually, however, these results have been obtained from 1, or in some instances from 2 or 3 injections of the arsenical. To determine whether the number of injections would play any rôle in the disposition of arsenic we have followed the quantity of arsenic present in the blood after serial administration of neoarsphenamine to dogs, maintained upon a constant fixed diet. The drug was introduced into either the jugular or femoral vein, and blood was drawn from an artery, usually of the leg. Each animal received 0.3 gram neoarsphenamine at intervals of 4 days. The arsenic was determined in 10 cc. samples of blood, by the combined methods of Chittenden and Smith, and Sanger and Black, the total quantity in the body being derived from a calculation of the blood volume from the body weight. The results are detailed in Table I.

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* References to the literature may be found in Kolmer, A. J., *Chemotherapy*, p. 538 *et seq.*

TABLE I.

Arsenic in Blood after Serial Administration of Neoarsphenamine.

DOG 1.

Dose injected, 0.3 gm. Neoarsphenamine (Av. weight, 15.5 k.)
(0.3 gm. Neoarsphenamine—66,000 mg. As.)

Injection	½ hr.	1 hr.	3 hr.	6 hr.	10 hr.	19 hr.	24 hr.	48 hr.	72 hr.
1st	9300	6975		Trace	Trace		Trace	0	0
2nd		6975	2325	1162			Trace	0	0
3rd	6975		2325	1162		Trace	0	0	0
4th	11625	9300	2325	1162			Trace	Trace	0
5th	4650	4000		1704				Trace	Trace
6th	6975			(5½ hr.)		Trace	Trace	Trace	Trace (65 hr.)

DOG 2.

Dose injected, 0.3 gm. Neoarsphenamine (Av. weight, 14.5 k.)

1st	4856	4260	1957	1065	0	0	0	0	0
2nd	4260	1065		1065			0	0	0
3rd	4260		1278	852			Trace	0	0
4th	4648		2130				Trace	0	0
5th	6390	5325	4260				Trace	Trace	0
6th	5964							Trace	0
7th	5325	2130	1800				Trace	Trace	0

All figures are given in micromilligrams of arsenic.

From the table may be seen, as others have found, a rapid disappearance of arsenic from the blood within the first one-half hour, after which the decrease is more gradual. It is to be noted that in the case of Dog 1 traces of arsenic can still be found at 24 hours following each of the first 2 injections, but that after the fourth injection there is still a trace present at 48 hours. Following the fifth injection traces can be found at 72 hours and at 65 hours after the sixth injection. With Dog 2 no traces of arsenic can be found after 24 hours after each of the first 4 injections, but after the fifth, sixth and seventh injections traces of arsenic can be shown at 48 hours.

In the case of Dog 1 after the fourth injection there is a much greater amount of arsenic in the blood after 30 minutes and 1 hour than there is at the corresponding time intervals after each of the first three injections. Also in the case of Dog 2 there is a slight increase in arsenic content after the fifth injection at all intervals as compared with the 4 previous injections.

It is quite evident from these data that with increasing injections of neoarsphenamine there is a slight but definite tendency toward a "lag" in the elimination of arsenic from the blood. The significance of this is problematical. Whether it means that the tissues absorb the arsenical less rapidly or that the renal function rate is decreased by possible injury remains open for future determination.

3738

On the Production of Immune Sera for Tissues.

K. LANDSTEINER AND J. VAN DER SCHEER.

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In continuation of studies on cell antigens we examined the production of immune sera for tissues. In the literature there are contradictory reports, but several authors demonstrated marked organ specificity of such sera, *e. g.*, Halpern,[1] Fleisher,[2] and recently Witebsky and Steinfeld.[3] We found that immune sera could be obtained very easily and almost regularly by intravenous administration of small quantities of cell or organ suspensions, *i. e.*, by 3 weekly injections of 10 mg. each of the fresh material suspended in saline. This was done with the hope that in this manner sera of higher specificity could be got than by the injection of larger quantities, as have been used generally. The following materials (ox) were taken as antigens: Tracheal epithelium, thymus cells, kidney and washed sperm cells. The resulting sera differentiated clearly in complement fixation tests between these 4 materials, an example of which is shown in the table.

To 0.5 cc. of progressively doubled dilutions of inactivated serum, starting with 1:10, was added 1 drop of an emulsion of about 2% of the antigen and 0.25 cc. of 1:10 guinea pig serum. After incubation for 1 hour at 37° C., 0.25 cc. of diluted Forssman immune serum containing 2½ hemolytic units and 1 drop of 50% sheep blood were added. The strength of the reactions is indicated as follows: 0 = no hemolysis, tr. = trace, d = distinct, w = weak, str. = strong, v. str. = very strong, ac. = almost complete, c = complete hemolysis.

TABLE.

Antigens	Immune sera for			
	Trachea epithelium	Thymus	Kidney	Spermatozoa
Trachea epithelium	0, 0, 0, 0, str, ac	d, tr, d, ac	str, v. str, ac, c	v. str, ac, c, c
Thymus	0, 0, d, ac	0, 0, 0, d, ac, c	v. str, ac, c, c	str, v. str, ac, c
Kidney	w, str, ac, c	ac, c, c, c	0,0,0,0,0,v.str,ac	d, str, v. str, c
Spermatozoa	ac, c, c, c	c, c, c, c	ac, c, c, c	0,0,0, tr, v.stra,c

On testing, however, with a large number (19) of other tissues group reactions were obtained, more frequently with some sera than with others, also in cases where there is neither histological nor embryological relationship, *e. g.*, trachea immune serum on brain. Only the sperm immune serum proved to be entirely organ specific. The immune sera were Wassermann negative and had moderate (kidney), little (trachea), or practically no (thymus, sperm) hemolytic activity for ox blood.

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[1] Halpern, J., *Z. f. Immunitätsf.*, 1911, xi, 609.

[2] Fleisher, M. S., Hall, T. G., and Arnstein, N., *J. Immunol.*, 1920, v, 437. Fleisher, M. S., and Arnstein, N., *J. Immunol.*, 1921, vi, 223. Fleisher, M. S., *J. Immunol.*, 1922, vii, 51.

[3] Witebsky, E., and Steinfeld, *Centralblatt f. Bakteriologie*, 1927, civ, 144.

3739

Composition of Bone. II. Analytical Results.

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The micro-technique which we[1] have devised for analyzing small specimens of calcified tissue has been subjected to further test and study. The crushed bones are extracted with ether and alcohol immediately after removal from the body; they are then dried and pulverized. About 10 mg. of bone powder are digested with HCl, trichloracetic acid is added, the solution is made up to 10 cc. and then filtered. Calcium is determined on aliquots of this filtrate. To another aliquot molybdic acid reagent (Briggs-Bell-Doisy) is added, the volume is made up to 10 cc. and then filtered. Inorganic phosphorus is determined on aliquots of this second filtrate. Digestion with HCl of different samples of the same specimen of bone powder gives solutions whose content of calcium and inorganic phosphorus agree as well as duplicate determinations on aliquots of the same solution.

CO_2 is determined on about 20 mg. of bone powder in Van Slyke's manometric gas apparatus.

A specimen of crushed bone was divided into two portions. One portion was extracted with ether and alcohol immediately, the other was allowed to stand unextracted during the summer. After standing for 5 months it was extracted and analyzed. The results for calcium, inorganic phosphorus and CO_2 were the same for both portions. Similar results were obtained with a second specimen of normal bone. The second precipitation with the molybdic acid reagent does not interfere with the accuracy of the method; the values for the ratio residual Ca/P so obtained are normal within the experimental error. When phosphorus values obtained from the first filtrate are employed, low ratios result.

In studying the method 11 specimens of normal calcification and 7 specimens of pathological calcification were analyzed. In all cases the value of the ratio was 1.96 within the experimental error; the theoretical value from $Ca_3(PO_4)_2$ is 1.94. One type of exception was noted. Three specimens of calcified fibroid of the uterus gave ratios of 2.23, 2.23, and 2.18 respectively.

The normal adult rats analyzed contained about 15% carbonate calcium, *i. e.,* carbonate Ca/total Ca × 100 was about 15. In the young rats analyzed the carbonate calcium was only from 8 to 10% of the total calcium. This indicates that the composition of bone in rats is not constant, but changes with age.

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[1] Shear, M. J., and Kramer, B., *J. Biol. Chem.*, 1927, lxxiv, 9.

3740

Immunization Against Pneumococcus by Feeding Desiccated or Milk Suspended Organisms.

VICTOR ROSS.

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The author previously demonstrated that white rats can be protected against many lethal doses of pneumococci, intraperitoneally injected, if these animals are first fed either the tissues of animals killed by this germ,[1] living pneumococci,[2] or HCL killed germs.[3] It was next decided to determine whether the acid killed germ when dried and mixed with cracker meal and the acid killed germ when still moist and suspended in milk could also be used. The object was to simplify the method of administering the germs as far as possible, consistent with successful immunization. At the same time success or failure accompanying a given modification gradually helps toward an explanation of oral immunization with the pneumococcus.

The organisms were grown, killed and centrifuged as described elsewhere.[3] A thin layer of germs was spread on a watch crystal and warmed on a water bath, so that they were at no time heated above 37° C. Desiccation was complete in 15 minutes. For 20 days each rat was fed the dried germs, 50 cc. of culture per day, mixed with moistened cracker meal. For testing the resistance of control and treated animals, a 24 hour virulent culture was diluted serially and the desired amount injected intraperitoneally in a volume of 0.20 cc. Table I contains the data for this experiment, and gives the rat number, whether control or experimental, its weight, the dose injected and the result. These data show clearly that the rats which were fed the dried pneumococci are considerably more resistant to the virulent germ than the control animals. The experiment was repeated, using larger rats, which responded less regularly than rats of the weights shown in Table I, although to the same degree.

TABLE I.

Effect of feeding desiccated, acid killed pneumococci (Type I) on the resistance of rats to intraperitoneal injection of the living germ.

Date 1926	Rat	Wt. gm.	Dose cc.	Result	Date 1926	Rat	Wt. gm.	Dose cc.	Result
Oct. 1	C	117	10^{-6}	D2	Oct. 11	C	166	10^{-5}	D2
	E	120	10^{-6}	S		E	115	10^{-6}	D4*
Oct. 4	C	114	10^{-8}	S		E	147	10^{-5}	S
	C	119	10^{-7}	S		E	143	10^{-5}	S
	C	130	10^{-6}	D2		E	143	10^{-5}	S
	C	132	10^{-5}	D2		E	163	10^{-4}	S
	E	120	10^{-6}	S		E	150	10^{-4}	S
	E	129	10^{-5}	S	Oct. 13	C	130	10^{-8}	D2
	E	124	10^{-5}	S		C	130	10^{-7}	D2
	E	132	10^{-4}	D3		C	132	10^{-6}	D2
Oct. 7	C	118	10^{-8}	S		E	143	10^{-5}	S
	C	124	10^{-7}	D2		E	130	10^{-5}	D2
	C	132	10^{-6}	D2		E	149	10^{-4}	D2
	C	148	10^{-5}	D2		E	147	10^{-4}	D2
	E	113	10^{-6}	S	Oct. 14	C	130	10^{-8}	D2
	E	135	10^{-5}	S		C	132	10^{-7}	D5
	E	139	10^{-5}	S		C	157	10^{-6}	D2
	E	137	10^{-5}	S		C	160	10^{-5}	D3
	E	141	10^{-4}	S		E	120	10^{-6}	D2
	E	146	10^{-4}	D4		E	120	10^{-5}	S
Oct. 11	C	120	10^{-8}	S		E	137	10^{-5}	S
	C	130	10^{-7}	D2		E	148	10^{-4}	D1
	C	137	10^{-6}	D2		E	155	10^{-4}	S

*Weak at the beginning. C = Control. E = Experimental. D = died-days. Last day of feeding germs, October 1. S = Survived.

In connection with this study of the effect of different methods of oral administration of germs, an experiment was done in which the freshly prepared, acid killed, centrifuged pneumococci were suspended in milk and given by medicine dropper directly into the mouths of the rats. For 19 days each animal received the bacteria from 50 cc. culture per day. Table II gives figures for this test. The results were excellent. The treated animals tolerated 1,000 to 10,000 fatal doses.

TABLE II.

Effect of feeding moist acid killed pneumococci (Type I) suspended in milk on the resistance of rats to intraperitoneal injection of the living germ.

Date, 1926	Rat	Wt. gm.	Dose cc.	Result
Nov. 12	E	128	10^{-5}	S
	E	107	10^{-6}	S
	C	167	10^{-5}	D2
	C	147	10^{-6}	D4
	C	125	10^{-7}	D2
	C	117	10^{-8}	D2
Nov. 13	E	136	10^{-4}	S
	E	107	10^{-5}	S
	E	113	10^{-5}	S
	E	104	10^{-6}	S
	C	130	10^{-6}	D2
	C	127	10^{-7}	S
	C	121	10^{-8}	S
Nov. 16	E	138	10^{-4}	S
	E	151	10^{-4}	S
	E	133	10^{-5}	S
	E	136	10^{-5}	S
	C	158	10^{-5}	D2
	C	144	10^{-6}	D2
	C	143	10^{-7}	D2
	C	142	10^{-8}	D2

C = Control. E = Experimental. D = Died-days. Last day of feeding germs, November 12. S = Survived.

As a further extension of these modified methods of the oral administration of the pneumococci, we shall study the effect of (1) dissolving the organisms in bile, feeding this mixture either in liquid form or after drying the whole, (2) mechanically disrupting the organisms both moist and dry, and, (3) enzymically digesting the bacteria. Up to the present, the use of moist, acid killed pneumococci, freshly prepared and mixed with cracker meal or suspended in milk, appears to be the most desirable of the modifications employed.

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[1] Ross, Victor, *J. Immunol.*, 1926, xii, 219-229.

[2] Ross, Victor, *J. Immunol.*, 1926, xii, 237-249.

[3] Ross, Victor, *J. Lab. and Clin. Med.*, 1927, xii, 566-572.

3741

Action of Picric Acid on Living Protoplasm.

HERBERT POLLACK. (Introduced by Robert Chambers.)

From the Laboratory of Cellular Biology, Department of Anatomy, Cornell Medical College.

Amoeba were injected with saturated aqueous and alcoholic (95%) solutions of picric acid. When an aqueous solution is injected in amounts equal to half the volume of the amoeba, no toxic effects can be seen. The amoeba is diffusely colored yellow, the intensity of color depending on the amount injected. If the injection be made so as to produce an injury at the point of insertion of the pipette, a local coagulation results. This coagulated portion is extruded by the amoeba, which then resumes normal activity and appearance, except for the yellow coloration.

When a saturated alcoholic solution is injected, there is a tendency for coagulation to occur. Unless an enormous amount is injected, the coagulated portion is localized, and is always extruded. The yellow colored amoeba then recovers completely. Hiller[1] has shown that an injection of small quantities of alcohol in concentrations above 80% will cause a local reversible coagulation. The picric acid dissolved in the alcohol apparently makes this coagulation irreversible.

These experiments indicate that picric acid solutions which definitely coagulate proteins in the test tube, have no such action on the interior of the living amoeba. However, if there is a local injury, the picric acid will act on the injured cytoplasm, resulting in coagulation.

In contrast to the non-toxicity to the internal healthy cytoplasm is the extreme toxicity of the picric acid when applied to the surface.

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[1] Hiller, S., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 427.

3742

Determinations of the pH of Developing Fundulus Eggs.

PHILIP B. ARMSTRONG. (Introduced by Robert Chambers.)

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By the use of a micro-pipette, solutions of Clark and Lubs' indicator dyes were injected into the subchorionic space, the pericardial cavity, and the yolk of developing Fundulus eggs, 4 to 6 days after fertilization, and the colorimetric pH values of the contents were determined. A few injections were also made into the brain vesicles. Single injections were made in most of the eggs. In several cases an injection was made into the subchorionic space, then the pipette was carried into the pericardial cavity, and another injection made in order to detect simultaneously any possible difference in the pH of different spaces in the same egg. The color tints of the indicators were always determined at the horizon of the egg in order to eliminate interference from the yellowish color of the yolk.

The color of the subchorionic space indicated a pH identical with that of sea-water. The critical indicators used were phenol red and cresol red, but the indicators above and below the ranges of these two were also injected. If the developing eggs were placed in distilled water for 18 hours and then injected, the pH of the subchorionic space was found to be that of the surrounding medium, *viz.*, 5.6±1. Controls in sea-water exhibited a subchorionic pH of sea-water. There was no leakage around the pipette at the point of puncture, because the outward diffusion of the injected dye occurred only through the membrane as a whole, and not around the pipette at the site of the puncture.

In his experiments on the permeability of developing Fundulus eggs to acid, Loeb[1] stressed the importance of placing the eggs in distilled water for 24 hours previous to the experiment in order to rid the membrane of adhering electrolytes. He found that more consistent results were obtained in the experiments performed on material so treated but, as pointed out above, such treatment changes the pH of the subchorionic space, a possibility he did not suggest.

The pH of the pericardial cavity was also found to approach that of sea-water. However, the pericardial cavity differed from the subchorionic space in that its pH did not change in eggs immersed in distilled water, nor did the dye diffuse out of the embryo. The tissue at the immediate site of puncture became yellow almost immediately when the phenol red or cresol red was injected, which was probably due to the acidity of injury, the importance of which has been stressed by Needham and Needham[2] and by Chambers and Pollock[3] in pH determinations on living tissues. There was, however, no outward leakage of the dye at the site of puncture. The few injections into the brain vesicles indicated that it had the same pH as that of the pericardial cavity. The pH of the yolk differed from that of the pericardial cavity and brain vesicles, as it was distinctly acid to cresol red and phenol red.

By the use of a micro hydrogen electrode Bodine[4] found the mean average internal pH value of the developing Fundulus egg to be 6.39. He did not, however, discuss the possibility of variations of pH between the different structures and cavities within the developing egg. His average probably represents some one constant sample which he always obtained by his technique, possibly yolk mixed with some subchorionic fluid which would be obtained in his procedure. In a differentiating organism it would seem preferable to study the pH of the several differentiated structures, rather than to attempt the average which would necessitate maceration with a resulting acidity of injury.

Since the subchorionic space changes so readily in pH with changes in pH of the environment but without change within the embryo, it is suggested that it is not at the chorionic membrane that the acid-salt antagonism takes place, as Loeb thought. Experiments have already been conducted on a large number of developing embryos from which the egg membrane has been removed, and it has been found that the cellular wall of the embryo and not the chorionic membrane is the site of the acid-salt antagonism.

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[1] Loeb, J., *J. Gen. Physiol.*, 1922-23, v, 231.

[2] Needham, J., and Needham, D. M., *Proc. Roy. Soc. London,* Series B, xcix, 173.

[3] Chambers, R., and Pollock, H., *J. Gen. Physiol.*, 1926-27, x, 739.

[4] Bodine, J. H., *J. Gen. Physiol.*, 1926-27, x, 533.

3743

Synthetic Ephedrine.*

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From the Department of Pharmacology, Johns Hopkins University.

Ephedrine was successively synthesized by Nagai,[1] Späth and Göhring,[2] and Eberhard.[3] The chemical difference between the ephedrine occurring naturally and that prepared synthetically lies in their optical activity, the natural being laevo-rotatory while the synthetic is optically inactive. From the pharmacological point of view, one would expect synthetic ephedrine to possess an action similar to that of the natural product but to be less potent; for it is known that the racemic form of several well known substances, notably hyoscyamine, hyoscine, homatropine, and epinephrine is weaker than the *l*-form.[4] After the recent demonstration of the action and clinical uses of the ephedrine isolated from the Chinese medicinal herb Ma Huang, investigators studied the synthetic ephedrine, and showed its comparable effects to those of the natural product.[5, 6, 7, 8, 9, 10, 11]

The present work consists of a comparison, both qualitative and quantitative, of the natural ephedrine prepared under the supervision of the author in 1924, and the synthetic ephedrine courteously supplied by E. Merck of Darmstadt, Germany. Its trade name is ephetonine. A preliminary clinical trial is also included. The hydrochloride of the synthetic compound melts at 187° C., is optically inactive, in aqueous solution at 21° C., and gives with cupric hydroxide a purple color extractable by ether,[12] It is apparently stable to light, air and heat.

When applied locally to the conjunctival sac of rabbits and man, synthetic ephedrine produces mydriasis with the preservation of the light reflex. An intravenous injection of the solution in the dosage of 2 mg. per kg. in anesthetized dogs, cats, or rabbits, results in a prolonged rise of arterial blood pressure, lasting for 25 to 30 minutes or longer, much the same as that seen after a similar injection of the natural ephedrine. It accelerates the pulse rate, especially after atropinization, and constricts the vessels of the kidney, the nasal mucous membrane and accessory nasal sinuses in dogs. Ergotamine reduces its pressor action, but does not invert it to a depressor action as it does with epinephrine. The synthetic ephedrine restores the respiration and blood pressure after large doses of morphine in anesthetized animals, and relieves bronchial spasm artificially induced by the intravenous injection of arecoline or physostigmine. It contracts the isolated guinea pig's uterus. It appears clear, therefore, that qualitatively it is similar in all respects to the natural ephedrine.

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Quantitatively, the pressor action of the natural and the synthetic ephedrine cannot be compared in the same anesthetized animal, for the initial injection of the one always overpowers the subsequent injection of the other. In man, a dose of 50 to 100 mg. of the synthetic ephedrine *per os* sometimes raises the systolic blood pressure by a few mm. of mercury, but is much less effective than an equal dose of the natural ephedrine given to the same subject on a different day. In Caucasians, the synthetic ephedrine appears to be a less powerful mydriatic than the natural, but the ratio of the increase of the transverse diameter of the pupil caused by a 10% solution of the former to that caused by a 10% solution of the latter in the same subject, calculated from a limited number of observations, has not exceeded 1.0:1.1. The M. L. D. of each drug in the form of the hydrochloride in white rabbits is 60 mg. per kilogram. Aside from the inequality of the pressor action in man after oral administration the difference of activity between the natural and the synthetic ephedrine does not seem to be great.

Clinically, the synthetic drug was given in 50 mg. dosage *per os* to several cases of bronchial asthma of mild or moderate severity. Most of the patients had relief from their attacks. In 4 cases of hypertrophied turbinates and 3 cases of acute rhinitis, a 5% solution of the synthetic ephedrine hydrochloride, applied locally, definitely caused a constriction lasting for 2 to 3½ hours. There were no signs of irritation or secondary congestion. It is hoped that by further comparative study, the synthetic drug may be proven to be clinically as useful as the natural ephedrine.

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3744

The Racial Difference of the Mydriatic Action of Ephedrines, Cocaine, and Euphthalmine.*

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In the continuation of the study of ephedrine as a mydriatic,[1] it was found that in Caucasians a 10% solution uniformly produces mydriasis by local application, and that its mydriatic action is further strengthened if it contains, in addition, 0.1% of homatropine hydrobromide, or 1.0% of euphthalmine hydrochloride. These solutions are useful in ophthalmoscopic examination, especially from the patients' point of view, because they cause almost no change in accommodation. There were no untoward symptoms or signs noted. Only in iritis and uveitis do they fail to dilate the pupil.

TABLE I.

A comparison of mydriasis occurring in different races, 1 hour after an instillation of 2 drops of the solution, observed in diffuse daylight.

Hydrochloride of	Strength of solution in per cent	Race	Number of observations	Mean increase† of the transverse diameter of the pupil in mm.
Ephedrine	10	Caucasian Chinese Negro	21 5 21	2.0+ 0.5+ 0.5
Pseudo-ephedrine	10	Caucasian Negro	20 21	1.0 0.0
Synthetic Ephedrine	10	Caucasian Negro	19 21	1.5 0.0
Cocaine	4	Caucasian Negro	20 21	2.0— 0.0
Euphthalmine	5	Caucasian Negro	20 21	2.5+ 1.0

* This investigation has been aided by a grant from the American Pharmaceutical Association Research Fund.

† Mean increase is the average of the central 3 or 4 figures of the data arranged numerically.

Ephedrine is, however, much less certain as a mydriatic in Chinese and Negroes. Not infrequently does it fail to produce any appreciable mydriasis. It was also found that pseudo-ephedrine, synthetic ephedrine, cocaine, and euphthalmine, are less powerful in dilating the pupil of the colored races. The results observed in diffuse daylight are summarized in Table I.

Further observations are being made under controlled illumination by accurately measuring the image of the pupil with a filar micrometer attachment in a telescope. These data corroborate the above preliminary measurements and will appear at a later date.

Howard and Lee of Peking[2] reported that ephedrine is more effective as a mydriatic in individuals with light irides than in those with dark. Their results are on the same line with ours.

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3745

Relation of Liver to Fat Metabolism. I. Respiratory Quotient in Conditions of Liver Insufficiency.

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It has long been known that animals fail to survive extirpation of the liver. The recognized changes in the blood resulting from this procedure show clearly that the liver is essentially concerned with both carbohydrate and protein metabolisms. Does the organ possess a vital function in relation to fat metabolism as well?

For the purpose of solving this and other problems, a successful method of inducing liver insufficiency in rabbits has been devised, whereby slightly more than 90% of the liver is removed without demonstrable interference with the return of blood to the heart. Unless treated, the animals survive but 6 to 18 hours, dying with low blood sugar and convulsions. If given glucose, they live for varying periods up to 5 days, dying eventually in a state resembling that developing in dogs deprived of the entire liver.[1] A study of the respiratory quotient of rabbits deprived of 90% of the liver has brought out the fact that fat metabolism is carried on as readily and as rapidly after the operation as before.

The respiratory quotient of intact rabbits, fasted 3 days, was found to be low, indicating that the body metabolism was largely that of fat. Fasting rabbits were, therefore, employed for the work and, after the preliminary determination of the respiratory quotient, 90% of the liver was ablated. Six to 8 hours thereafter, when the immediate effects of operation and anesthesia had worn off, the respiratory quotient was again determined. No glucose had been given and at this time the blood sugar concentration was found to be 0.60 to 0.70 mg. per cc. In several instances another respiratory quotient was determined on the following day, 24 hours after operation. The animals in which this was done received just enough glucose by mouth to maintain the blood sugar level above the minimum compatible with life.

All the respiratory quotients were obtained by the closed circuit method, the animals remaining in the respiration chamber 1½ hours for each determination. Only those instances have been considered in which the metabolic rate remained constant before and after operation. As a further precaution, the CO_2 content of the venous blood was determined just before and immediately following the sojourn of the animal in the respiration chamber. All experiments have been ruled out in which any change in the concentration of blood CO_2 was found. The findings given below must be attributed to the true gas exchange of the animal and not to a mere retention or blowing off of CO_2 during the periods of the experiments.

The preliminary respiratory quotients of 10 fasted rabbits averaged .750, with variations between .710 and .780, indicating a considerable fat consumption. When over 90% of the liver of these animals was ablated, the respiratory quotient 6 to 24 hours later was found to average .735, with individual variations between .714 and .761. Respiratory quotients taken again the following day were always slightly lower than those taken 6 to 8 hours after the operation.

Control experiments with freshly fed rabbits upon a carbohydrate diet showed respiratory quotients close to unity before and after removal of 90% of the liver. In rabbits receiving intravenous injection sufficient glucose to maintain the blood sugar level at normal, the respiratory quotients also approximated unity. This latter finding is in accord with that reported by Mann[2] for hepatectomized dogs receiving glucose administrations. The observations were repeated, with the same precautions, on dogs fasted 24 hours and then deprived of the "main liver," about 70% of the organ. Animals with respiratory quotients of .797, .731, .739, and .752 showed 8 to

24 hours later quotients of .728, .724, .752, and .790 respectively. The experiment was also repeated on rats fasted 24 hours, but with them the blood CO_2 analyses were omitted. In 5 animals the preliminary respiratory quotients were .785, .782, .765, .731, and .756 respectively before the operation, and, .723, .747, .741, .738, and .744, 24 hours after removal of 70% of the liver. In 3 other rats, on which no preliminary tests were made, respiratory quotients of .736, .739, and .749 were found 8 to 24 hours after ablation of a similar percentage of the liver.

In the past, experimenters have sought to ascertain the rôle of the liver in fat metabolism by respiratory quotient studies made after attempted exclusion of the organ from the circulation by ligation of the abdominal aorta and other vessels.[3, 4] Since the respiratory quotient was usually found close to unity, conclusions were drawn that the presence of the liver was essential to fat metabolism. Murlin, Edelmann and Kramer[5] have shown, however, that extensive interference with the circulation brings about changes in the blood CO_2 which alone may account for the results mentioned. They point out, too, that in the rare instances in which the animals survived more than 6 hours, much lower respiratory quotients were found. One may conclude from the findings reported above that the metabolism of fat is carried on adequately in animals deprived of over 90% of the organ and eventually dying of extreme liver insufficiency. It seems highly improbable that the liver possesses any vital function in fat metabolism.

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3746

The Mucinate Content of Saliva.

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(Introduced by W. J. Gies.)

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The method used for the determination of the mucinate content was the nephelometric procedure described by Inouye,[1] as follows: A 10 cc. sample of saliva is made alkaline with 2.5 cc. of 5% NaOH, thoroughly mixed, and promptly filtered through a dry filter. Five cc. of the filtrate, in a collodion bag, is dialyzed against running water until no longer alkaline to phenolphthalein (but still alkaline to litmus), and then against several changes of distilled water. The contents of the bag are then transferred to a 50 cc. volumetric flask and diluted to volume, of which 20 cc. are then treated with 0.02 N HCl from a burette, drop by drop, until an additional drop fails to give more precipitate. This is then compared nephelometrically with a standard solution of Na mucinate solution prepared as follows: A 10 cc. sample of 0.1% Na mucinate solution is dialyzed (if the mucinate is completely soluble in water without the addition of NaOH, the dialysis is omitted), and made up to 50 cc., of which 20 cc. are then titrated to maximum turbidity. If the standard and unknown give widely divergent readings, appropriate dilution of one of them should be made to bring the readings closer together.

The mechanical stimulation of chewing paraffin was used to obtain the "controls". Twenty minutes after the end of collection of a control sample, the mechanical stimulus was again applied or a chemical stimulus with a few drops of lime juice or crystals of rock-sugar on the tongue (in these cases the first expectorations were discarded), or the smoking of a cigarette, and a new sample collected for analysis. Twenty-six samples, from 9 subjects, under the mechanical stimulation, were analyzed. The subjects, "average" adult males, were selected with no reference to dental conditions. All samples were collected in the early afternoon, after lunch. The mucinate content ranged from 0.60% to 0.21%, with an average of 0.27%, with a P. E. of 0.05. If, however, we exclude one sample of 0.60% (which, though from an apparently "average" subject, may be an abnormally high mucin content) we then have a series of 25 samples, ranging from 0.29 to 0.21%, averaging 0.26% with a P. E. of 0.02. This, we believe, can be taken as a normal value of the mucinate content following the mechanical stimulation under these conditions.

The largest variation in the mucinate content of the samples from a given individual was 0.08%; subject W, 0.22-0.25%, five tests; subject H, 0.25-0.29%, three tests; subject F, 0.25-0.28%, six tests; subject C, 0.21-0.29%, two tests. Repetition of paraffin stimulation 20 min. after collection of the first "control" sample gave, in 2 of 6 cases, decreases of notable amounts (0.08%); but in 2 cases there was a decrease of only 0.01%, and in 2 case an increase of 0.03%.

Collection of samples under lime juice stimulation, 20 min. after the "control" collection, gave a decrease in mucinate content in each of 6 cases (the decrease amounting in 4 cases to as much as 0.10%). This accords with the frequent observation of a "watery" saliva after acid stimulation, perhaps due to parotid stimulation. In a short series (3 cases) cigarette smoking gave no consistent change in the mucinate content. In 7 tests in which sugar ("rock candy") was used following the control, there was a consistent decrease in the mucinate content. The maximum fall was not determined, in 4 cases the dilution made the nephelometric comparison unreliable. This, it should be noted, followed stimulation by small amounts of sugar, and does not contradict the common observation of an increase in the muciginous quality of saliva following the action of large amounts of sugar.

As a partial check on the results, the total N content of cleared and dialyzed saliva was determined in many cases. The values obtained showed the same trend and magnitude of change under varying stimuli, and corroborated the mucin amounts on the assumption that the protein present was largely mucinate.

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[1] Inouye, J. M., 1924, Studies of Salivary Mucin and its Quantitative Determination (Dissertation, Columbia University).

ERRATUM.

"Advanced Development of Some Echinoid Plutei," by A. R. Moore. Vol. XXV, No. 1, p. 38, 4th line from top of page, instead of "formed in 10 to 12 days, and in 10 days the third pair of arms ap-" to read, "formed, and in 10 to 12 days the third pair of arms ap-".